An Experimental and Clinical Evaluation of the use of Acid-Citrate-Dextrose (A.C.D.) Blood for Coronary Artery Perfusion and Extracorporeal Circulation.

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I N D E X

A P P E N D I X

INTRODUCTION

The almost universal acceptance of acid-citrate-dextrose (A.C.D.) solution as the anticoagulant-preservative mixture for blood transfusion is a tribute to its general suitability and safety. In specific situations where large volumes of blood relative to recipient blood volume have to be transfused, and particularly if the transfusion has to be rapid, the safety of A.C.D. blood has been questioned. Various substitutes e.g. heparin, Edglugate-Mg (Smith et al., 1959; Smith, Brown and Eadie 1962), ion exchange resins (Walter et al., 1951; Nealon et. E., 1960) citrate-phosphate-dextrose (C.P.D.) solution (Gibson et al., 1957; Schechter and Swan 1962), Ringer Gelatin Solution (Cookson et al., 1954) have been proposed as the blood anti-coagulants preferable in those circumstances in which A.C.D. blood would appear to be dangerous. This thesis presents the results of an effort to render A.C.D. blood safe for transfusion in those otherwise hazardous situations. Particular attention was paid to the special case of extracorporeal circulation for open-heart surgery.

The study comprises four main parts.

1. A Historical review of the literature.
2. An Evaluation of the Relative Importance of hypocalcaemia, Acid-Base Disturbances, Potassium and Temperature in producing disturbances of Cardiac Function during Acid-Citrate-Dextrose (A.C.D.) Blood Transfusion.
3. An assessment of the feasibility and safety of using "Converted" A.C.D. blood for cardio-pulmonary bypass.
4. Clinical use of "Converted" A.C.D. blood for cardiac bypass.

CHAPTER I

Historical Review

That Blood transfusion is a dangerous form of therapy was recognised by the pioneers of direct donor to recipient-transfusion by syringe techniques (Ziemssen, 1894). But the early hazards were those of Landsteiner and Rhesus group incompatibility, premature coagulation of the withdrawn blood before it could be administered, and uncertainty as to what volumes were required - or even how much had been given when the donor was connected by direct-vessel anastomosis to his recipient! (Carrel, 1902). The first transfusions rarely exceeded 500 ml. in volume, often occupied as much time as the operation they were meant to supplement, and were made with fresh unadulterated blood with its normal complement of electrolytes and coagulation factors.

The general adoption of the citrate method of anticoagulation and storage introduced in 1915 by Lewisohn in this country and Agote in Buenos Aires, removed the uncertainties about volumes replaced, established reliable anticoagulation, and paved the way towards larger transfusions from multiple donors.

The credit for being first to use sodium citrate as anticoagulant for blood prior to transfusion is handed magnanimously, if perhaps a little tongue-in-cheek, by Lewisohn to Hustin in Brussels (1914) who had given 150 ml. of citrated blood, diluted with an equal volume of dextrose solution, to a patient some months prior to the original publications by Lewisohn himself and Agote. (Lewisohn, 1915; Agote, 1915).

In the original method only enough trisodium citrate was added to maintain fluidity of the blood long enough to carry out the simple direct cross-matching of the times before administration to the recipient. Longer storage periods, but maintaining viability of the red cells at the acceptable level of 80% after 21 days were achieved by refrigeration at 4-6°C and modifications of the anticoagulant fluid along the basic lines elucidated by Loutit, Mollison and Young (Mollison and Young, 1942; Loutit et al., 1943; Loutit and Mollison 1943; Loutit, 1945). The solution suggested by Loutit and Mollison in 1943 has been in use with minor variations in Britain ever since. It has a pH of 5 and the following composition:-

Disodium hydrogen citrate	2G
Dextrose Anhydrous	3G
Water to	120 ml.

This is slightly different from the N.I.H. solution B, commonly used by the blood transfusion services of America, which contains trisodium citrate 1.32G, Citric Acid 0.48G, Dextrose 1.47G made up to 100 ml. in water. American A.C.D. blood, therefore, contains 16.36 mM/per litre of citrate compared with 15.6 mM/per litre in British banked blood. The addition of citric acid (or acidic sodium citrate) and dextrose was found greatly to enhance red cell survival at the expense of reducing the blood pH to 7.0 or less immediately after withdrawal (e.g. Nealon T. F., 1960; Schechter et al., 1962), causing a small amount of immediate red cell destruction (Strumia 1956, Gibson 1959) and temporarily diminishing the oxygen carrying capacity (Valtis and/

and Kennedy 1954).

Better storage properties led to the general development of blood banking facilities so that today many millions of units of acid-citrate-dextrose (A.C.D.) blood are used yearly throughout the world and, perhaps just as significantly, multilitre transfusions have become commonplace. Large and rapid transfusions (in relation to the blood volume of the patient) are particularly seen during resuscitation from hypovalaemic shock, in cardiac surgery using an extracorporeal circulation and in exchange transfusions for erythroblastosis in infants.

In transfusions under these circumstances the electrolytic and other changes occurring in stored blood may assume an importance which is not apparent in the slower or limited transfusions which account for the vast majority of clinical blood donations. Deleterious effects on cardiovascular function associated with or resulting from massive A.C.D. blood transfusion began to be recognised about 1943. As will be seen later five factors (citrate intoxication, diminished ionic calcium, acid-base disturbances, hyperkalaemia and reduction of body temperature) have been inculpated in the production of cardiac dysfunction during massive blood transfusion and it is a surprising fact that after over 40 years of experience with citrated blood transfusion there is limited basic knowledge of the relative importance of these factors.

The toxicity of trisodium citrate was recognised long before it was in wide use as an anticoagulant for blood transfusion. In rabbits 0.5G/Kg given subcutaneously produced tremors, while 0.96G/Kg led to the rapid onset of dyspnoea and death. With intravenous injections smaller doses (0.35G/Kg) were toxic and it was recognised even then that the speed of administration was an overwhelmingly important factor. A steady infusion of 50-70 mg. of

of trisodium citrate/Kg/min. regularly gave respiratory and/or cardiac arrest in both dogs and rabbits (Salant and Wise 1916). This is equivalent in citrate content to approximately 20 ml/Kg/min. of citrated whole blood, a rate of transfusion never dreamt of in these early days. The bradycardia which accompanies hypotension caused by large doses (50 mgm/Kg/min.) of intravenous citrate in dogs was found to be a direct action on the myocardium since section of the vagi or atropine injection did not accelerate the heart (Love 1923). Some influence of trisodium citrate on the central nervous system was found by the same author when he recorded increases in systemic blood pressure, and respiratory depression after initial stimulation, after intracarotid injection of an isotonic solution of the salt. Tetany, sluggish or absent eye reflexes and respiratory depression have also been noted experimentally (Adams et al., 1944). Whether or not these pharmacological actions were due to the alkaline pH of the infusate was not clarified but the experiments did emphasise the need for assessment of the effects of citrate solutions on the intact animal before drawing conclusions potentially referable to the human situation.

So far all experiments had been carried out on normotensive animals. With the increasing use of citrated blood for transfusion, interest was directed towards the assessment of citrated plasma and blood for resuscitation after exsanguination. In a preparation carrying an 84% mortality after massive single haemorrhage citrated autotransfusion was only marginally better (50% mortality) than saline infusion (58% mortality) and citrated plasma was considerably worse (70% mortality). Heparinised plasma was almost universally successful/

successful in restoring blood pressure and reduced mortality to 6% (Ivy et al., 1943). The addition of calcium salts during citrate infusions was not described by Ivy. The idea that the calcium ion was important in the aetiology of these circulatory derangements was clearly in the minds of Bruneau and Graham (1943) when they issued a "caution against the too liberal use of citrated blood in blood transfusion". Elevations of the total serum calcium in dogs dying after large reinfusions of blood collected into sodium citrate solution were noted, and may have dissuaded these authors from attempting to reverse citrate effects by giving further calcium. That larger quantities of citrated blood - up to 200 per cent. of the total blood volume - could, in fact, be rapidly transfused if calcium was given simultaneously was first demonstrated and clinically applied by Adams and his co-workers (1944). It was commented, however, that perhaps excessive caution was being shown since it required citrate sufficient to anticoagulate 60% of the blood volume given in 10-12 minutes to lead to death of a (normotensive) animal. This was equivalent to 4000 ml. of A.C.D. blood in five minutes to a 70 Kg. man "which would be practically impossible to duplicate clinically". The heart lung machine with its high blood flows and large priming volumes had not been foreseen.

Cookson and his co-workers (1954) repeated the findings of Ivy et al., (1943) and demonstrated the universal survival of exsanguinated dogs by the volumetric replacement of 44% of the blood volume with heparinised blood compared with a 92% mortality when citrated blood was similarly used. This work was widely used as a final argument for the need for heparinised blood for/

for extracorporeal circulation.

Cumulative effects of citrate as well as the rate of its administration were found experimentally to be important. Working with dogs which were being perfused at "high" flows (400 ml/minute) on the artificial kidney with A.C.D. blood Nakasone et al., (1954) noted precipitous decreases in blood pressure and ventricular stroke volume, variable changes in peripheral resistance, and E.C.G. changes recognised by subsequent investigators as those of diminished calcium ion concentration. Nevertheless by keeping the rate of administration at 0.02 mM/Kg/min. (one third of what Adams (1944) had shown to be lethal in 20 minutes) they were able to inject a total dose of approximately 1.8 mM of citrate/Kg. in 90 minutes. This corresponds to the giving of about 7 litres of A.C.D. blood in an adult. There was "..... an inverse ratio between the rate of intravenous infusion of A.C.D. solution and the cumulative amount of citrate required to produce equivalent E.C.G. changes ....".

Initially, therefore, in spite of much experimental evidence of cardiovascular and central nervous system toxicity of large doses of citrate, the safety of well cross-matched transfusion was never doubted since transfusion of more than 2 units was a rarity and the rate of administration seldom exceeded 500 ml. in half-an-hour. These quantities and rates were well within the normal capacity of the body to deal with by rapid metabolism of citrate in the liver (Howland et al., 1955), muscles (Howland et al., 1957) and kidney (Martensson 1940), excretion by the kidney as a calcium salt complex (Chang and Freeman, 1950; Weidner and Clowes, 1959), equilibration throughout the extracellular space (Bunker et al., 1955) and chemical combination with calcium mobilised/

mobilised from the bones (Saffran and Denstedt 1951). In fact an adult can metabolise as much as 40 grammes of sodium citrate per day after ingestion (Howland et al., 1957).

Reports of "citrate toxicity" in clinical practice began to appear in the nineteen-fifties (e.g. Ames et al., 1950; Bunker et al., 1955; Firt and Hejhal 1955; Howland et al., 1955; Hubbard et al., 1956; Bunker 1956; Howland et al., 1956; Clowes et al., 1957; Wexler et al., 1959). These recorded sudden death during blood transfusion, with cardiac arrest or ventricular fibrillation, which was not explicable on the basis of anoxia or failure of cardiac venous return; or reported hypotension persisting in spite of adequate volume replacement, but responding promptly to calcium administration. Interestingly much more resin-decalcified blood than A.C.D. blood could be exchange-transfused without electrocardiographic changes, although the calcium ion concentration in both kinds of blood was similarly low. The construction placed on this odd fact was that there must be a specific "citrate toxicity" unrelated to low ionic calcium concentration. If this were true it would be clinically relevant. However, haemodynamic and electrocardiographic responses to the infusion of the calcium chelating agent sodium ethylene diamine tetra acetate (EDTA) were similar to those seen during citrate infusion at similar depressions of calcium ion concentration (Bechtel et al., 1956; Surawicz et al., 1959). It is well to remember that A.C.D. blood contains an excess of citrate over that required for anticoagulation and this excess may remain potent to lower calcium ion concentration locally on reaching the myocardium after intravenous infusion.

One/

One of the constant practical difficulties in the study of citrate toxicity and its relation to calcium metabolism has been the absence of a simple, reliable and rapid method of measuring calcium ion concentration. The isolated frog heart preparation attached to a lever recording system gives reproducible responses to changing calcium ion concentration and has enabled the construction of nomograms relating total plasma calcium, plasma citrate concentration and plasma proteins to calcium ion concentration (McLean and Hastings 1934, 1935). The derivation of calcium ion concentration by this means, however, is cumbersome at best and liable to errors magnified by the variability of each individual measurement. The number of methods (e.g. Lewis and Melnick 1960; Woolen and Walker 1959; Baron and Bell 1959; Tisdall 1923) for assaying total plasma calcium alone testifies to the inaccuracies of earlier methods. One should accept with reserve, therefore, conclusions based on calculations of calcium ion levels.

With this proviso the observations on citrate intoxication by Bunker and his co-workers are of interest (Bunker et al., 1955; ~~1962~~: Bunker 1956). No evidence of toxicity was found in clinical transfusions when 500 ml. or less of A.C.D. blood were given to adults in 30 minutes or more, when citrate levels never increased above 9.0 mg% (normal 0.17 ± 0.08 mM/l in children (Ames 1950); 0.8 ± 0.8 mg% in males, 1.08 ± 0.9 in female adults (Howland et al., 1957)) and the ionised calcium remained above 0.85 mM/per litre. Massive evidence of citrate intoxication was lacking in spite of the discovery of very high plasma citrate levels (20 mgm% on 2 occasions and 47 mgm% in 1 other cirrhotic patient). Combinations of several factors appeared/

appeared to be necessary to produce the clinical syndrome of hypocalcaemia - i.e. failure to restore blood pressure with adequate volume replacement, but rapid clinical improvement when intravenous calcium was exhibited. The data were rather poorly organised to answer the question as to whether citrate toxicity is a function of calcium ion concentration, though many of the factors leading to high plasma citrate levels were elucidated. Foremost amongst these were hypothermia, which has received much more attention recently (Boyan et al., 1961, 1962; Boyan 1964), cirrhosis of the liver (but not partial resection or metastatic disease of the liver - Howland 1957) and operations on the hepatic veins and thoracic aorta (Bunker 1956).

The isolated normal liver, perfused with huge doses of citrate, effects almost complete clearance after a single passage of the perfusate (Howland 1955). The kidney is another important eliminator of the citrate ion both by oxidation and excretion (Martensson 1940; Weidner and Clowes 1959). Thus one can explain why cirrhosis, aortic clamping and operations on hepatic veins, involving as they do, partial or complete obstruction to the blood flow through the liver, limit the ability of the body to metabolise citrate and may lead to hypocalcaemia. The reduction of general metabolism may be enough to account for the high citrate levels and diminished calcium ion concentrations found during citrated blood transfusion in hypothermic dogs (Hara et al., 1961).

Further experimental support for the hypothesis that hypocalcaemia was fundamentally important in the causation of the sporadic cases of otherwise unexplained cardiac arrest associated with massive or ultra-rapid blood transfusion/

transfusion came from the work of Firt and Hejhal in Czechoslovakia (1955, 1956, 1957). Some new details of the cardiovascular effects of citrate came to light. Trisodium citrate by intravenous infusion was noted to produce acute right heart strain with blanching of the lung parenchyma, increasing central venous pressure, increased pulmonary arterial resistance and ultimately failure of contraction of the ventricle. Pulmonary vasoconstriction was the last effect to disappear spontaneously as citrate effects wore off, but all effects could be reversed by the administration of calcium salts. Using calcium gluconate, transfusion at 50 times the rates used previously and 16 times "the permissible rates" (Watkins 1953) for intra-arterial infusions, could be achieved. Ultra-rapid transfusions were shown to be desirable in combating so-called irreversible shock (Firt and Hejhal, 1956). Heparinised blood could restore normal blood pressure in shocked dogs (B.P. 25-40 mm. Hg. for 70 min.) when replaced at 6 ml/Kg/min. but not at lower rates. Unaltered A.C.D. blood when given rapidly caused circulatory overloading by the mechanisms mentioned above. High transfusion rates could be achieved with A.C.D. blood only by giving large doses of calcium gluconate intravenously into a separate vein whenever central venous pressure tended to increase. A rather empirical scheme for covering blood transfusion was worked out and used clinically (Firt and Hejhal 1955).

Large transfusions even though not particularly rapid can also be dangerous. Tetany, electrocardiographic abnormalities and deaths have occurred, and were reported during exchange transfusions in erythroblastotic infants/

infants (Wexler et al., 1959). It has become routine practice since then to give calcium prophylactically during these exchange transfusions with marked reduction in cardio-vascular complications.

A conflicting view of the importance of hypocalcaemic citrate intoxication in clinical cardiac arrest was held by Howland. (Howland et al., 1957) After describing the occurrence of ventricular fibrillation in 9 patients who received more than 2.5 litres of blood rapidly, including one who developed tetany with gross hypocalcaemic E.C.G. changes (Howland et al, 1956) there was an abrupt volte face when in a further series (Howland et al., 1957) failure to observe citrate intoxication was reported. However, their second series of patients were a heterogenous group receiving variable quantities of blood at variable rates for a variety of different conditions and states of anaesthesia which must make analysis considerably less precise than in the experimental animal. No cases of tetany or persistent hypotension were seen though E.C.G. changes such as prolongation of the corrected Q-T interval were mentioned and it is noteworthy that the patient with the highest plasma citrate level (173 mgm%) was given 20G of calcium gluconate during treatment. It was concluded that calcium administration during blood transfusion is not necessary unless shock is present since sufficient calcium is available for mobilisation as long as bone blood flow is maintained.

Up to this point the position appeared quite clear therefore. In certain unusual circumstances, involving rapid or very large transfusions with citrated blood, the normal calcium transfer mechanisms become overwhelmed and circulatory embarassment occurs similar to that reproducible experimentally by diminishing the calcium ion concentration. It was a fine/

fine simple concept, which simply did not explain all the facts. Alternative or additional explanations of these tragedies of resuscitation were put forward by LeVeen (1959) and Howland's group at the Sloan Kettering Hospital in New York. It was long known (Duliere 1931) that potassium tended to accummulate in the plasma of stored blood even in the absence of haemolysis. The loss of the "sodium pump" in the stored cells allows intracellular potassium passively to diffuse out of the cell - a process which can be reversed by rewarming the blood to 37°C for 6 - 8 hours (LeVeen et al., 1959) when adequate glucose is present for cellular metabolism. Since rapid or large intravenous injections of potassium salts were known to be cardiotoxic (Zwemer and Scudder, 1938) it was suggested that here lay the explanation of some of the sudden deaths which occurred during massive blood transfusion. The increased sensitivity to potassium in the hypotensive dog was demonstrated (LeVeen et al., 1959). Infusion of potassium chloride solution containing 400 mEq/L was equally lethal to animals rendered hypotensive, to 80 and 110 mm. mercury, at 37 per cent of the dose which produced cardiac arrest in normotensive animals. Since the hypotensive animals had a reduced circulating blood volume and a diminished cardiac output this difference is explicable by the shorter time taken to reach the lethal level of circa 35 mEqu/L. Digitalis by increasing cardiac output was able to prolong the period of potassium equilibration with the extravascular space, and thereby to increase the tolerated dose of potassium. It was concluded from these data that the routine use of digitalis in patients about to undergo major surgery when serious blood loss is anticipated might prevent "transfusion arrest". A recommendation to use calcium also as/

as a potassium antagonist was also made, but without factual evidence to support it. The combination of sodium citrate anticoagulant solution at 4.5 ml/min. to a total of 25 ml/Kg and potassium chloride solution (400 mEqu/L) at approximately 4-5 ml/min. was tested in 3 normotensive dogs and was found to be somewhat more toxic than potassium chloride infusion alone. This interesting observation was not pursued further, in spite of the fact that the citrate infusion was only equivalent to one unit (500 ml.) of A.C.D. blood every 25-30 minutes - well within the rates encountered clinically. The potassium infusion at 2.0 mEqu/minute (my figures since there are inconsistencies in the text), was comparable with the infusion of three-week-old blood at five times this rate. The conclusion from this widely quoted work, admittedly on rather flimsy evidence, was that hyperkalaemia was a factor of over-riding importance in producing cardiac arrest during massive blood transfusion. Support for this view has been limited by the recognition of the dubious clinical significance of raised plasma potassium levels in agonal conditions (Winkler and Hoff, 1943) and by the difficulties in obtaining central venous, atrial, or arterial blood samples - the only ones which accurately reflect the potassium content of the blood perfusing the coronary arteries. Heart blood potassium concentrations of up to 8 mEqu/L have been reported but would receive scant support as a cause of cardiac arrest from the experimental or clinical evidence (Winkler et al., 1938; Miller et al., 1954; LeVeen et al., 1959).

In the years subsequent to 1959 emphasis gradually shifted to the multifactorial aetiology of clinical cardiac arrest (e.g. Gain, 1962). Often quite clearly/

clearly the metabolic alterations normally present in banked blood or plasma proved to be the final straw to a heart previously weakened by anoxic insults, anaesthetic drugs, haemorrhagic hypotension with its consequent poor coronary and general systemic perfusion, mechanical outflow obstruction as in pulmonary embolism, or intrinsic metabolic disorders in the patients themselves. Papers still appeared slanted towards one or other of the factors which appeared to the authors to be of new clinical or experimental importance. The influence of acid-base disturbances (Baue et al., 1961; Schweizer and Howland 1962; Howland and Schweizer 1962) and hypothermia (Boyan and Howland 1961; Boyan and Howland 1962; Boyan 1964; Hara et al., 1961) were extensively discussed. The difficulty in coming to valid conclusions based on clinical material is exemplified by the writings of Howland and his group (1961-1964). It is characteristic of the clinical problem that differences between results in groups of patients can easily receive explanations other than those immediately apparent. For example, Howland (1961) analysed the results of transfusion in 266 patients who received more than 5 units of citrated blood. Of these, 114 were given calcium salts during therapy and 152 received no calcium. 21 patients who received calcium were given 16 units of blood or more and 10 died, compared with 1 death in 6 who received no calcium and the same volume of blood. When high rates of flow were compared, it was found that 12/57 patients receiving calcium died, while only 3/69 died when no calcium was given. In many ways the series were not comparable and were not concurrent. For example, there was a preponderance of smaller (5-7 unit) transfusions/

fusions in the non-calcium group (93 against 42) and conversely, as one can see from the figures quoted, more patients received calcium when large volumes of blood were given. It is also pointed out that "calcium may have been given as a last desperate measure of therapy to sustain life", i.e., the calcium was not given in a regulated way during the transfusion by a scheme such as that recommended by Firt and Hejhal (1956), or Moore (1960) and as can be done in a controlled trial or experiment. The conclusion that calcium, if not actually deleterious (an idea insinuated by the text), was not advantageous, is not warranted on this kind of evidence, yet has important clinical implications if true.

Similarly, the beneficial influence of rewarming blood prior to transfusion claimed by Boyan et al., (1964) cannot be due purely to heat transference alone, since a simple calculation will show that 10 litres of blood at 10°C given to a 70 Kg man, could lower his temperature no more than about $4\frac{1}{2}$°C even in the total absence of heat production by the patient himself. The cooling of a patient actually produced by cold blood transfusion must, therefore, inevitably be much less than this amount. The gross degrees of hypothermia (27°C-29°C) noted by Boyan and his colleagues must have been associated with prolonged anaesthesia using muscular relaxants and with excessive heat losses from thoracotomy or laparotomy wounds in addition to the effects of cold blood infusion.

One of the early results of the recognition of the cardiac toxicity of A.C.D. blood, particularly to cardiac surgical patients (e.g. Hubbard et al., 1956)/

1956) was the avoidance of A.C.D. blood in cardio-pulmonary bypass work and the employment of fresh heparinised blood instead. This policy necessitated special sessions for the withdrawal of the blood. In many places this was done on the morning of a scheduled operation and required special arrangements to be made for cross-matching before the pump oxygenator could be primed. Particularly in the United States of America where much of the pioneer work in open cardiac surgery was done, the need for blood for priming extracorporeal circuits grew apace in the late nineteen-fifties. By 1963 there were, for example, 17 teams performing heart surgery in the Los Angeles area alone (Krumhaar et al., 1963). This placed a heavy strain on the blood collection resources in addition to being inconvenient, expensive and wasteful. Since blood banks licensed by the National Institute of Health in the United States may not employ heparinised blood for routine use, all unused heparinised blood in excess of that needed to prime the pump oxygenator and to cover operative blood losses was frequently discarded at the end of the operative procedure.

It is not surprising, therefore, that search was made for a suitable substitute for fresh heparinised blood. The search took several directions. The period of acceptibility of heparinised blood itself was extended by the addition of dextrose which appears to maintain normal red cell metabolic activity and a normal plasma potassium for approximately 24 hours (Abbott et al., 1958). Smith and his co-workers (1959) introduced Edglugate-Mg., a new anticoagulant preservative which acts, as does citrate, by lowering the/

the ionic calcium level in blood. To convert it for use in extracorporeal circuits, heparin and calcium chloride solutions were added just prior to priming the circuit. Similar conversion of A.C.D. blood was rejected at this time because Bunker (1955) had stated that the amount of calcium needed was very unpredictable - *in vivo*.

Blood decalcified by passage over an ion-exchange resin (Chollett *et al.*, 1959) could be similarly "converted" before use and the citrate in A.C.D. blood can actually be removed by means of passage of the blood over Dowex-1 x 8 an ion exchange resin in the bicarbonate cycle (Nealon T. F., 1960; Shechter *et al.*, 1958). One group of authors (Cookson *et al.*, 1954) went so far as to separate the red cells from the plasma and resuspend them by a rather complex series of manoeuvres, in Ringer-Gelatin solution.

None of these solutions of the problem have proved entirely satisfactory either from the practical or the theoretical angle. Any departure from the routine practice of the blood bank is liable to introduce difficulties. These would be acceptable if the new anticoagulant mixture provided equally good preservation of red cell viability and coagulation factors as does A.C.D. solution and was non-toxic. The usually accepted criteria of satisfactory red cell metabolic activity and preservation are the rate and extent of increase in the plasma potassium and plasma haemoglobin, the decrease of blood glucose with refrigeration storage of banked blood, and the *in vivo* survival of radio-isotope tagged cells. With A.C.D. stored blood as the standard of comparison none of the methods of preservation of blood/

blood mentioned above compare favourably in all these respects (Smith et al., 1959; Schechter et al., 1962; Schechter and Swan 1962; Emerson 1950; MacGovern et al., 1959).

The beauty of A.C.D. solution as an anticoagulant is that citrate and glucose are normal metabolities, sodium is usually readily handled by the kidneys and the water load of even a huge transfusion merely represents the daily water requirements of the patient. If, therefore, standard A.C.D. stored blood could be rendered suitable for the rapid infusion rates occurring at the onset of total body perfusion by cardio-pulmonary bypass a considerable advantage might be gained.

CHAPTER 2.

An evaluation of the relative importance of hypocalcaemia, acid-base disturbances, hyperkalaemia and cold on cardiac function in the dog.

CORONARY PERFUSIONS

Even if one accepts the published evidence on the whole as showing that citrate linked to diminished ionic calcium concentration, hyperkalaemia, acid-base disturbances, and hypothermia in A.C.D. blood may all play a part in increasing the risk of cardiac dysfunction associated with large and rapid A.C.D. blood transfusion, particularly to the shocked, oligaemic, cirrhotic or hypothermic patient, one can hardly fail to realize that the importance of these factors relative to one another has received scant attention.

In order to dissociate effects of electrolyte infusion on the heart itself from effects on the peripheral vasculature a method of intermittent perfusion of the coronary arteries was employed in dogs. It is to be emphasised that these studies were carried out on the normally-innervated intact heart.

Use of the same experimental preparation for all assessments allows a comparison of the relative magnitudes of the changes produced by coronary perfusion with blood containing widely differing ionic contents of calcium, hydrogen and potassium.

GENERAL METHOD FOR ALL CORONARY ARTERY PERFUSIONS

Anaesthesia

Mongrel dogs weighing 6.3 to 20 Kg were anaesthetised with intravenous pentobarbital sodium (30-35 mg/Kg) and a cuffed endotracheal tube inserted and inflated. Intermittent positive pressure respiration with air was maintained by means of a Palmer veterinary respirator pump set at 19 cycles per minute. The stroke volume of thepump was set at a level of 100 to 200 ml., roughly proportional to the size of the dog and judged to give good inflation of the lungs when the chest was opened. Further small doses of

of pentobarbital occasionally had to be given during the course of the experiment.

Position.

The dogs lay in the supine position, with the chest rotated towards the right and with the left forelimb extended cephalad. E.C.G. leads were connected by means of needle electrodes to the limbs, and to the V_4 position opposite the apex of the heart.

Operative Procedure

Clean but non-sterile technique was used throughout. Small incisions were made in both groins to expose both femoral arteries and the right femoral vein: On the right side both vessels were cannulated with No. 8 F.G. Portex cannulae (Cat. No. B.205: O.D. 2.76 mm.). A three-way stop-cock was attached to each cannula. The venous cannula was used for administration of drugs such as heparin and sodium bicarbonate solution, as indicated by the experimental protocols, and for the maintenance of anaesthesia. The arterial cannula after connection by rigid polythene tubing to a pressure transducer (Bell and Howell type 4-326-L212), allowed both recording of central aortic pressure and the withdrawal of arterial blood samples for electrolyte and acid-base measurements. The left femoral artery had two ligatures placed in position, but left loose at this stage.

A thoracotomy through the 4th left intercostal space was made using diathermy for careful haemostasis. The appearances and principal anatomical features are shown in Fig. I which demonstrates the chest open with the left lung retracted inferiorly to expose the mediastinal structures. An incision Fig. 2 (1) was made in the pleura overlying the origin of the brachio-cephalic artery, posterior to the phrenic nerve, and extended upwards to

to expose its primary division into left common carotid and innominate arteries. The innominate was ligated at its origin and the carotid artery was prepared for cannulation. Several small vessels require ligation during this dissection.

A small incision Fig. 2 (2) was now made in the pleura in the concavity of the aorta between the vagus and phrenic nerves. By gentle blunt dissection a curved artery forceps was passed upwards deep to the aortic arch till its tip appeared in the space between the brachio-cephalic and left subclavian arteries. This maneouvre allowed the exact and easy placement of a vascular clamp across the aorta during perfusion of the coronary arteries.

The pericardium was widely opened along a line parallel to and anterior to the left phrenic nerve (Fig 2 (3)) revealing the left auricular appendage and the anterior ventricular surfaces. A 120 ohm Walton-Brodie brass-encased strain gauge arch (Figs. 4, 5 and 6) was sutured to the surface of one of the ventricles. The right ventricle was used except in the case of the hypothermia experiments. Attention was paid to the following details during the placement of the device.

A. Deep bites of ventricular muscle were taken with a 16 mm half-circle atraumatic needle carrying 2/0 black thread (Ethicon No. 333). This ensured that cutting out of the thread would not occur during the course of the experiment.

B. Care was taken to avoid puncture of coronary arteries during insertion of the ventricular stitches. The exact direction on the ventricular surface in which the long axis of the strain gauge is placed is of no

no importance (Cotten et al., 1953).

C. Once the two stay sutures had been inserted in the ventricle, the distance between them (usually approximately 15 mm) was determined. The metal feet were adjusted so that the holes in them lay one and one half times this distance apart. This process ensured that, when the sutures were tied firmly into the feet of the bridge, the entrapped muscle segment which was monitored as a representative portion of the entire ventricular syncytium was stretched by approximately 50 per cent. (As the degree of myocardial stretch increases from 30 to 60 per cent some increase in the recorded force of contraction occurs. The percentage change in ventricular contractile force (V.C.F.) due to a given stimulus remains constant, however, at different degrees of stretch within this range (Cotten, 1956)).

D. The movable foot was firmly fixed by means of its brass screw before final attachment of the device to the heart. (It has been determined that percentage change in ventricular contractile force (V.C.F.) is the best method of expressing results obtained with this instrument. Loosening of the screw would lead to lossof stretch of the ventricular segment and to changes not only of the absolute values of V.C.F. but also to unreliability in measurements of percentage change of V.C.F.)

The electrical leads of the Brodie bridge, elongated by the addition of 2 metres of 2-core light-weight screened cable, were connected to the three fixed arms of a matching balanced Wheatstone bridge (Fig. 5). Variations in the internal resistance of the Brodie bridge, produced by,

by, and directly proportional to, the force of ventricular contraction applied to its two feet, lead to imbalance of the Wheatstone bridge and, therefore, to flows of current between pins 2 and 3 of the Wheatstone bridge which can be amplified and recorded.

A Devices' 8 channel recorder was employed throughout this study for simultaneous monitoring of V.C.F., central aortic blood pressure and Leads II and V4 of the electrocardiogram. The sensetivity of the Devices Amplifier System (D.C.2) for the V.C.F. channel was set at a level giving a 10 to 20 mm. deflection with each cardiac contraction, prior to the first coronary arterial perfusion. Thereafter, no further adjustments were made. No measurements of absolute force applied to the feet of the Brodie bridge were made. Each E.C.G. Channel was calibrated to give a 10 mm. deflection to a 1mV input. The blood pressure channel was calibrated by means of a mercury manometer to give a full scale deflection recording 0-200 mm. of mercury. The calibration was checked frequently between perfusions but it is very stable with this recording apparatus. For all measurements a paper speed of 2.5 cm./Sec. was used (1 mm. = 0.04 sec.).

The dog was now given 3 mg./Kg of heparin by intravenous injection. A Bardic 28F atrial cannula was inserted into the left auricular appendage through a small incision. A purse-string suture of 2/0 black silk, was tightened and anchored to the cannula after checking that the tip of the cannula lay in the atrium (Fig. 4). The largest possible arterial cannula (No. 10-14 F.G.) was inserted into the distally ligated left femoral artery and anchored securely in place. The two cannulae were filled with blood by gently releasing their clamps and then connected together by means of

of polyethylene tubing (Baxter Total Body Perfusion Pump Set No. U311) previously completely filled with heparinised normal saline. A left ventricular bypass could then be achieved by inserting the Baxter pump set into the tube track of a variable flow, totally occlusive, rotary pump (Watson-Marlow Type H.R.E.) and pumping blood from left atrium to femoral artery.

A Portex cannula (No. 8 F.G., O.D. 2.76 mm., Cat. No. B205) was inserted into the distally ligated left carotid artery, after ligature of the innominate artery at its origin, and manipulated so that the tip lay in the brachiocephalic trunk. In order to achieve this cannulation the carotid artery was often transected. The continuation of spontaneous breathing movements and contracted pupils after this procedure indicate that some cerebral circulation still remained. Fig. 7 shows the left heart bypass and brachio-cephalic arterial catheter in place. A curved atraumatic aortic clamp was manipulated into position (Fig. 3) and the preparation was then ready for the first coronary arterial perfusion.

Before any perfusions were done the acid-base status of the animal was assessed and any severe degree of metabolic acidosis resulting from the anaesthesia and manipulations was corrected by the intravenous administration of 8.4% sodium bicarbonate solution using the Astrup formula:- Volume of sodium bicarbonate solution (1m.Equ/ml) required = 0.3 x Body Weight in Kilograms x Base excess (mEqu/L).

The Perfusions

After a control recording of V.C.F., systemic blood pressure and the two E.C.G. leads (II and V4) the rotary pump of the left heart bypass was

was started,(Fig. 8.). The speed of rotation of the pump was adjusted until the pump tubing began to collapse signifying that the entire left atrial return was being bypassed to the femoral artery, perfusing the aorta retrograde. The aorta was then cross-clamped with the atraumatic vascular clamp just distal to the origin of the brachio-cephalic trunk,(Fig. 9.) These procedures isolate the proximal aorta, a fact which could be checked by attempting to withdraw blood by means of the brachio-cephalic catheter. If the left heart bypass was properly adjusted only a few millilitres of blood could be rapidly obtained and the aorta then felt empty.

The injection of fully oxygenated citrated or heparinised blood at a known measured temperature was then started (Fig. 10). A standard volume of 50 ml. was infused in exactly 60 seconds. Various additions of electrolytes were made to the blood prior to injection and samples were taken for estimation of pH, PCO_2, Standard Bicarbonate, Base Excess, haematocrit, plasma sodium, potassium and total calcium. The temperature of the blood was measured by means of a clean mercury thermometer before anaerobic transfer of a sample to another syringe.

Records at a paper speed of 2.5 cm/sec. were made at the start of perfusion and every 15 seconds during perfusion. When the injection of 50 ml. blood was completed (Fig. 11) the aortic clamp was removed and the pump was stopped in rapid succession. Further tracings at 2.5 cm/sec., 1 minute and 2 minutes post-perfusion were made to demonstrate the return of the preparation to control conditions once normal circulation was restored (Fig. 12).

A typical example of the type of tracing obtained is shown in Fig. 13.

13. This was a perfusion employing oxygenated heparinised blood at 37°C. The phases of the record (control period 0,15, 30, 45, 60 seconds of perfusion, release of the aortic clamp and stopping of the pump after perfusion, and the demonstration of return to control levels 1 and 2 minutes post-perfusion) are noted beside the tracings.

The information to be gained from the tracings was abstracted in tabular form, along with the measurements of electrolytes levels in each perfusate. The numerical analysis from which the evaluations of the effects of calcium, acid-base alterations, hypothermia and hyperkalaemia on coronary perfusion were made, are presented in Tables 16 - 43 in the Appendix.

In analysing the records the following conventions were followed.

1. Individual perfusions were identified by the experiment number (1-15) and the sequential number of the perfusion in that experiment. Thus the first perfusion of experiment seven was designated 7 (1) and so on.
2. Contractile force measurements were made from the trough of one contraction to the peak of the next. Where pulsus alternans was present both figures were given. Where only minor variations in amplitude occurred from beat to beat the mean of the wave amplitudes available (usually 5-6) was taken.

The mean amplitude of the control period V.C.F. tracing was in each perfusion taken as 100%. The measurement at 30 seconds and 60 seconds of coronary perfusion was then calculated as a percentage increase or decrease on the control figure. See Fig. 14 (Perfusion 5 (6)). In this example the V.C.F. during control period was recorded as 10mm. and that at 60 seconds of perfusion was 8mm. V.C.F. at 60 seconds of perfusion No.5 (6) was then calculated as -20%

as -20%. . This has been shown (Cotten 1953) to be the most reliable method of expressing results obtained with the Walton-Brodie strain gauge arch.

The mean V.C.F. figure two minutes after the end of perfusion was also recorded as a check on the completeness or otherwise of the recovery of the preparation from what was sometimes a highly "toxic" perfusion.

3. The height of the T-wave in Lead II was recorded as a positive or negative quantity in millimetres relative to the isoelectric line. The difference between the T-wave after 30 or 60 seconds of perfusion (T30; T60) and the T-wave during the control period of the same perfusion was used as one measure of the effect of the perfusion on the E.C.G.

4. The Q-T interval was measured in millimetres during recording at a paper speed of 2.5 cm. per sec. (5 mm = 0.2 sec.). The changes in Q-T after 30 and 60 seconds of each perfusion were derived by comparison with the control Q-T, as with other parameters, and used as the second measure of the effect of perfusion on the E.C.G.
Many measurements of other E.C.G. parameters (heights of P waves, P-R intervals, heights of R-waves) were made in the first few experiments but are not recorded in the detailed tables of the data of the experiments, since no changes were apparent in them.

5. Changes in heart rate during coronary perfusion were also recorded. They are reported as an (+) increase or (-) decrease in beats per minute over the control rate e.g. +30/120. In comparing changes of rate in different perfusions percentage changes were used. Thus a

a decrease of 30 beats per minute from a control rate of 120 would in comparisons be converted to -25/100.

6. In some records for reasons of electrical interference, fluctuation of baseline or the presence of ventricular extrasystoles and other such arrythmias, measurements of electrocardiographic complexes would be valueless. These spaces are marked in the data tables, "--". Extrasystoles are annotated "E.S." and electrical alternans, sometimes with alternately upright and inverted T-waves, is annotated "Alt.". Ventricular fibrillation, a rare occurrence, is contracted to"V.F.".

7. The figures given for PCO_2 were obtained in mm. of mercury with the microelectrode Astrup apparatus at 38°C and have been corrected for temperature to that of the animal or perfusate from which the blood sample was obtained, by means of the line diagram of Bradley et al., 1956.

8. pH measurements were made at 38°C and also were corrected for temperature to that of the animal or perfusate from which they were obtained. The formula of Adamsonswas used for this correction (Adamsons, 1964).

9. Standard bicarbonate and base excess figures, derived from pH measurements at 38°C with the Astrup apparatus and the nomogram of Anderson and Engel (1960), are in mEqu/l. These, by definition, do not vary with the temperature of the original sample.

10. Plasma sodium and potassium were measured by means of a flame spectrophotometer.

11. Plasma calcium was measured by the method of Bett and Fraser, 1959.

THE EFFECT OF CALCIUM ON CARDIAC CONTRACTILITY DURING CORONARY PERFUSION WITH A.C.D. BLOOD

Introduction

The effects of acid and citrate infusions on blood pressure in the intact animal are well documented. Some of the most relevant findings have been mentioned in the historical review and need no repetition here. The pure effects of citrate on the function of the heart are less completely ascertained and their reversal by calcium has not been quantitatively assayed, except in the frog (Hastings et al., 1934). The coronary perfusion preparation described in the previous section allows the measurement of the short-term changes on cardiac contractility produced by adding varying amounts of calcium to A.C.D. blood perfusing the coronary arteries in the dog.

THEORETICAL CONSIDERATIONS.

The composition of the A.C.D. solution used in the Aberdeen Blood Bank is as follows:-

Disodium hydrogen citrate, B.P.	2.4 Gm.
Hydrous Dextrose	3.0 Gm.
Water for injection	120 ml.

This volume is made up to 540 ml. with venous blood to give a citrate concentration in banked blood of 16.9 mm. per litre - somewhat higher than the 15.6 mM/L which is common in British practice.

Bunker and his co-workers (Bunker et al., 1955) estimated calcium ion concentrations during massive citrate infusions in man and found that marked cardiac depression was accompanied by the lowering of the calcium ion concentration from the normal 1mM. per litre to less than 0.85 mM per litre. (Coagulation is prevented by a calcium ion concentration of 0.5 mM per litre or less). It would appear, therefore, that a calcium ion level of about 1 mM per litre is necessary for adequate cardiac action in the presence of a normal plasma potassium concentration and pH. The ionisation of calcium citrate has been determined (Hastings et al., 1934). By using these authors' nomogram relating total citrate, total calcium and calcium ion concentration, one finds that to maintain a calcium ion concentration of 1 mM per litre in the face of the 16.9 mM per litre of citrate present in Aberdeen A.C.D. blood the total calcium must be increased to 10.5 mM per litre. Since normal total blood calcium is about 2.5 mM per litre, the added calcium should, therefore, be about 8.02 mM per litre. Thus on theoretical grounds the additional calcium must be of the order of 11.8 ml. of 20 per cent calcium chloride solution B.P. (0.68 mM of calcium per millilitre) /

litre) per litre of A.C.D. blood. It should be emphasised that the ionised calcium represents only a small proportion of the total plasma calcium. For example, reference to the Hastings-McLean nomogram indicates that the addition of sufficient calcium to citrated blood to raise total calcium from 2.5 to 11.8 mM per litre increases calcium ion concentration only to 1.3 mM per litre. Conversely it requires only the addition of 7.6 mM of calcium per litre to bring ionised calcium to 0.85 mM per litre which, according to Bunker and his associates, is a level adequate for normal myocardial function. This calcium citrate ionisation system should in theory serve as an excellent "buffer" to give wide latitude in the restoration of acceptable calcium ion concentration in A.C.D. blood.

Extension of the above type of calculation reveals the results quoted below.

Calcium Chloride 20% B.P. (ml) Added to 540 ml.	A.C.D. Blood 1000 ml.	Total Citrate mM/L.	Calculated Total Calcium mM/L.	Calcium ion Conc. mM/L.
4.0	7.4	16.9	7.5	0.5
5.3	9.9	16.9	9.2	0.7
6.0	11.2	16.9	10.1	0.85
6.4	11.8	16.9	10.5	1.0
7.5	13.7	16.9	11.8	1.3
5.7	10.6	15.6	9.7	1.0
6.2	11.5	16.3	10.3	1.0
	12.9	18.2	11.32	1.0

≠ Blood from Aberdeen Blood Bank (120 ml. A.C.D. Solution in 540 ml.).

† Blood from other British Blood Banks (120 ml. A.C.D. Solution in 540 ml.).

Δ Blood from American Red Cross Regional Blood Center, Los Angeles.

o Dog blood with 120 ml. Aberdeen A.C.D. solution in 500 ml.

These figures are given in order to allow ready computation of values related to their own blood samples by others interested in the controlled recalcification of A.C.D. blood. Note the differences in calcium addition which will be required by users of different A.C.D. solutions in order to give equivalent calcium ion concentrations in the final product.

THE EFFECT OF CALCIUM ON CARDIAC CONTRACTILITY DURING CORONARY PERFUSION WITH A.C.D. BLOOD

Method

Dog's blood containing 120 ml. A.C.D. solution (Aberdeen Blood Bank Solution) made up to 500 ml. was obtained from a separate donor dog immediately before each coronary perfusion experiment. Heparinised blood (2,500 units heparin per 500 ml.) was also withdrawn from the same donor. In each of 8 dogs several perfusions with A.C.D. blood containing heparin (5,000 units/L to maintain anticoagulation) and various additions of calcium chloride solution 20% B.P. ranging from 0 to 1.6 ml. per 50 ml. of blood, were carried out. These were compared with perfusions with heparinised blood. All perfusates were warmed to 37°C in a water bath, were 50 ml. in volume, and were injected evenly over 60 seconds. It was usual to carry out one heparinised blood perfusion first and thereafter the A.C.D. perfusions followed in order of diminishing expected toxicity. A further heparinised blood perfusion was usually interspersed amongst the A.C.D. perfusions. There was an interval of fifteen to twenty minutes between perfusions to allow the preparation to recover, and for equilibration of added electrolytes to take place.

The tracings were analysed in the manner previously described and the percentage changes in V.C.F., changes in T-wave and changes in Q-T interval of the E.C.G. after 60 seconds of perfusion, were tabulated. The effect of calcium on percentage change in V.C.F. produced by coronary perfusion with A.C.D. blood are abstracted from the general tables in Table 1. For each preparation a graph was drawn of the tabulated figures for calcium and percentage change of V.C.F. A best fit of the points was drawn assuming a

a linear relationship between calcium addition and change in V.C.F. in the range 0.2 to 1.6 ml. of calcium chloride addition. These lines are illustrated in miniaturised form in Figs. 18 and 19. On each line the point corresponding to the change in V.C.F. seen with heparinised blood perfusion in that particular dog preparation was plotted. This one may call the heparin equivalence point. It represents the quantity of calcium chloride 20% solution B.P. which, when added to 50 ml. of A.C.D. blood, produces the same effects on cardiac contractility as heparinised blood, during coronary perfusion in dogs.

Results

Fourteen heparin equivalence points were plotted in 8 experiments. The mean quantity of calcium chloride solution B.P. 20% required to produce heparin equivalence in 50 ml. of A.C.D. blood was 0.686 ml. (Range 0.35 - 0.88 ml. : Standard deviation ± 0.13).

This quantity compares quite closely with the amount calculated to give a calcium ion concentration of 1 mM. per litre in dog's A.C.D. blood, tabled in the introduction to this section.(12.9 ml. per 1,000 ml. blood equals 0.645 ml. calcium chloride solution 20% B.P. per 50 ml.)

It will be seen from Table 1 that as calcium addition to A.C.D. blood was increased, percentage change in V.C.F. decreased; i.e. cardiac contractility was less depressed when calcium was added to the blood prior to perfusion than when A.C.D. blood without calcium addition, was used. An example of a perfusion with A.C.D. blood, to which only heparin had been added, is given in Fig. 15. In the latter case (Perfusion 3 (7)) there was a catastrophic depression of V.C.F. after 60 seconds perfusion by 94% of the

the control V.C.F. The heart at this point was dilated, tense and could be seen to be merely twitching feebly, only to recover rapidly once normal circulation was restored at the end of the period of perfusion. Such a series of events was invariable when unrecalcified A.C.D. blood was the perfusate.

When calcium chloride solution in excess of the amount required to produce heparin equivalence was employed, large increases in V.C.F. were seen, e.g. perfusions 1 (3) and 4 (11). It should be noted that the addition of calcium was capable of overcoming any depression of myocardial contractility which might be attributable to the acid component of the acid-citrate-dextrose complex. All the perfusions quoted in Table 1 were of A.C.D. blood modified only by the addition of calcium chloride and without acid-base correction.

The effects on the electrocardiogram of the addition of calcium to A.C.D. blood before coronary perfusions was also analysed. There was a general trend towards an increase in T-wave and a decrease in Q-T interval when the calcium chloride was 0.4 ml. per 50 ml. of A.C.D. or greater. Gross S-T segment depressions and elongations of the Q-T intervals were seen when no calcium was added to A.C.D. blood (Fig 15) Hypercalcaemic E.C.G. patterns were seen when 1.6 ml. of calcium chloride solution were added to a perfusate.

Heparinised blood perfusions produced minimal disturbances of the E.C.G. In 21 perfusions in dogs 1-12 there was an average decrease of 1.5 mm. in the T-waves and no significant change at all in the Q-T intervals. The E.C.G.'s. from A.C.D. blood perfusates containing 0.6 ml. of calcium chloride solution in 50 ml. most closely resembled the corresponding

corresponding E.C.G.'s. of their heparinised blood counterparts.

Beyond these generalisations, more detailed conclusions are not justified from the data contained in Tables 16 to 43.

Discussion

The addition of heparin to maintain anticoagulation while the calcium ion concentration was increased to normal levels by the addition of 0.686 ± 0.13 ml. of 20% calcium chloride B.P. was shown to "convert" 50 ml. of A.C.D. blood to equivalence with heparinised blood as far as their effects on myocardial contractility and electrocardiographic patterns were concerned. Reversal of cardiac depression could be achieved despite a lack of correction of the respiratory and metabolic acidosis normally present in stored citrated blood.

These results emphasise that there is a fairly narrow range of calcium chloride addition which will produce heparin equivalence in A.C.D. blood and clearly demonstrate the severe myocardial depression produced when unaltered A.C.D. blood perfuses the heart. A linear relationship was found between calcium addition to A.C.D. blood and the percentage change in contractile force produced by such blood during coronary perfusion, once any improvement in V.C.F. was discernible at all.

Reference to Figs. 18 and 19 will show the points at which the V.C.F. regression lines, when extrapolated, cut the ordinate. There was a variation, in different dog preparations, from 0 (in experiment 3) to 0.55 (in experiment 2.). These points represent the theoretical quantity of calcium below which, in that particular dog, 100% depression of myocardial contractility might be expected. The reason for this variation is not

not immediately obvious but may merely be that individual myocardial sensetivity to citrate is a widely variable entity.

The fact, however, that heparin equivalence could be achieved only within the narrow range described above, eliminates individual variation as an important factor in determining the quantity of calcium required for "conversion" of A.C.D. blood to a suitable fluid to replace heparinised blood.

At this point in the investigation, therefore, it seemed probable that the addition of heparin and calcium to A.C.D. blood, in quantities similar to those producing equivalence to heparinised blood in coronary perfusion, might be all that was needed to render banked blood safe for whole body perfusions at high flow rates.

The fact that large doses of calcium salts would be used in the large priming volumes of some heart-lung machines did not preclude the use of converted A.C.D. blood. The clinical toxicity of calcium (Hoff et al., 1939; Wall 1939; Clarke 1941) is real, though transient - producing flushing of the face, palpitations, oppression of breathing, nausea, a metallic taste in the mouth, profuse perspiration, dull headache, an increase in systolic blood pressure, decreased diastolic pressure, bradycardia and sometimes transient partial or complete heart block. Previous clinical tests were, however, lacking the balanced antagonism of citrate seen here. More than 25 mEqu. of calcium per litre, mostly in ionised form, was found experimentally to be toxic in the dog, (Hoff et al., 1939). However, it must be remembered that the calcium ion concentration in recalcified citrated blood at the heparin equivalence point is, in theory,

theory, at least, only 1mM per litre. It is believed that only ionised calcium is important in determining cardiac contractility and toxicity.

Before describing the experimental and clinical use of "converted" A.C.D. blood for cardio-pulmonary bypass it is convenient and relevant to present in the ensuing three sections of this chapter, observations on the effects of acid-base alterations, hyperkalaemia and hypothermia on myocardial function as demonstrated by the coronary perfusion technique. These factors have all been credited with the major place in the cardiac toxicity of A.C.D. blood during massive blood transfusions both clinically and experimentally. An assessment of their relative importance is of interest particularly if old blood, with an increasing potassium and metabolic acid content, was to be contemplated for use in pump surgery, or if hypothermia was deliberately to be induced by blood cooling during perfusion.

The adequacy of the heparin anticoagulation in converted A.C.D. blood was also investigated before clinical application. The results of these experiments are reported in the ensuing chapter.

ACID-BASE

It seems appropriate, before introducing the subject of the effects of acidosis on cardiac function, to digress a little to general considerations regarding the recording and measurement of acid-base data. Up till 1956, accurate measurement of acid-base alterations in blood was a clinical rarity though a research accomplishment. It was a procedure in most hands invalidated by apparatus incapable of recording pH values more accurately than to the nearest 0.05 units, by inadequate or absent temperature control of the electrodes and of the samples, but above all by failure of the investigators themselves to recognise (1) the importance and possibility of the dissociation of respiratory from metabolic components of acidosis, (2) the need for standardisation and statement of the temperature of measurement of pH and PCO_2, and (3) the care required to obtain and maintain blood samples free from contact with air or other equilibrating gas, before a pH reading is made.

The work of Severinghaus and his associates (Severinghaus *et al.*, 1956; Bradley *et al.*, 1956) had shown the rate of change of pH in blood samples left at room temperature for varying lengths of time and anyone who had used an open pH electrode was aware of the upward "drift" of pH if a sample was measured during exposure to air. Changes in pH (Δ pH) of the order of 0.0147 units in blood and 0.013 units in plasma per $1^{\circ}C$ change in temperature were shown to occur when the carbon dioxide content was at or near normal and kept constant prior to measurement (Rosenthal 1948). The alterations which take place in response to temperature change are a little more complex than Rosenthal imagined and have recently been further elucidated (Adamsons

(Adamsons et al., 1964). It would appear that as packed cell volume (P.C.V.) decreases below 20%, Δ pH per 1°C in blood approaches that in plasma. Δ pH also varies with plasma CO_2 content but not with the degree of oxygenation. The factor for correction of pH for temperature is -

$$\Delta \text{ pH} = 0.0146 - 0.005\ (7.4 - pH_{38}) + 0.005\ (20 - CO_2)$$

where pH_{38} is the pH measured at 38°C without change in the CO_2 content (CO_2) from that present when the specimen was obtained.

PCO_2 also varies with temperature. As temperature is lowered the solubility of carbon dioxide increases (Severinghaus et al., 1956; Austin et al., 1963) and, when free facility exists for equilibration with a fixed supernatant carbon dioxide concentration, the carbon dioxide content increases. Blood equilibrated with 5 per cent carbon dioxide at 10°C for example, must have a PCO_2 of approximately 36 mm. mercury, if measurement is made at 10°C. A sample of this blood sealed and rewarmed to 38°C will tend to release some of its dissolved carbon dioxide and will develop an increase of PCO_2 to approximately 100 mm. of mercury. The carbon dioxide being unable to escape will remain dissolved in the plasma and ionise partially into H^+ and HCO_3^- thus accounting for some of the pH alteration already mentioned.

Introduction of the term "standard bicarbonate" is credited to Astrup and his co-workers (Astrup 1956; Jorgensen and Astrup 1957; Astrup et al., 1960; Andersen and Engel 1960; Mellemgaard and Astrup, 1960; Andersen et al., 1960; Andersen 1961). This term alone has revolutionised, by simplification, the determination of the relative importance of respiratory

respiratory and metabolic aspects of acidosis in any particular case. Although Astrup's work was preceded by that of Brewin and his associates, in Britain in 1955 - and gives scant recognition of the fact - the now almost universally used nomenclature and nomogram, introduced by the Scandinavians, are important landmarks in the evolution of rational acid-base studies and therapy.

These considerations are presented in some detail since they explain the precautions taken in measuring and reporting the acid-base data on the blood used for coronary perfusion. All measurements were made as soon as possible after withdrawal of specimens. Where there was even a short delay the samples were kept refrigerated until assayed. pH and PCO_2 were measured by means of the Astrup (Radiometer) micro-electrode apparatus at 38°C and corrected for temperature using the Adamsons formula and the PCO_2 line diagram of Bradley (Bradley et al., 1956).

The increasing practice of open heart surgery in the late nineteen fifties using pump-oxygenators, many of the earlier of which required large volumes of blood to prime them, reopened a host of problems relating to massive transfusion for investigation. Great interest centred around the acid base alterations which were found to occur if low perfusion rates were used. The concept that acidosis resulted from inadequate total body perfusion was elaborated by many authors who also correlated its persistence in the post-operative period with myocardial insufficiency. In a typical series comparing normothermic perfusions in dogs at high (70-110 ml./Kg./min.), medium (40-60 ml./Kg./Min.) and

and low flow rates (26-36 ml./Kg./min.), lactic acid accummulation during perfusion was found to be inversely proportional to flow rate (Clowes et al., 1958). Post operative metabolic acidosis was seen to be most marked in those that died. The mortality went hand-in-hand with the degree of acidosis. Intravenous infusion of hydrochloric acid in the dog was shown to result in hypotension, electrocardiographic changes similar to those of potassium infusions (Roberts and Magida, 1953) respiratory depression after initial stimulation and ultimately cardiac arrest or fibrillation (Harkins and Hastings, 1931). Lactic acid infusion could produce similar changes (Clowes et al., 1958). A direct depressive effect of acidosis on the myocardium was suspected. The evidence of diminished contractile force, measured by a Walton-Brodie strain-gauge arch, in response to metabolic acidosis (Darby et al., 1960) confirmed that this was an important mechanism. Earlier work on isolated guinea pig hearts exposed to acidotic perfusion fluids demonstrated that this was a phenomenon affecting other species of mammal besides the dog (McElroy et al., 1948).

Ringer, whose perfusion solutions have made his name a byword, made a great number of fundamental observations on the influence of blood electrolytes on the contraction of the frog heart (Ringer, 1882). Crude though his apparatus was, it is interesting to compare what he said then with these more recent observations. Briefly, he said that acidity in the bathing fluid caused weakening of the contractions while sodium car-:bonate brought about arrest in systole : sodium bicarbonate was able to

to improve contractions particularly if calcium was present.

Human blood when freshly drawn into A.C.D. solution has a pH of approximately 7.0 and its PCO_2 is usually in excess of 150 mm. of mercury. Base excess as estimated on the Astrup Scale is less than -22 mEqu./L., the lower point recorded on the scale. Dog's blood has an even lower initial pH (6.30 to 6.60). Thus the immediate effect of A.C.D. blood transfusion is to create an acidosis both metabolic and respiratory. Carbon dioxide is rapidly excreted by the lungs. The metabolism or excretion of citrate is so rapid in the liver (Howland et al., 1955) kidney (Martensson, 1940; Chang et al., 1950; Weidner et al., 1959) and muscles (Bell et al., 1961) and the buffering systems of the extracellular space are so efficient, that very large quantities of A.C.D. plasma or blood can be given with only a transient decrease in blood pH (Moore, 1959). Furthermore, the metabolism of citrate leads ultimately to the formation of carbon dioxide and water by the citric acid cycle (Bell, Davidson and Scarborough 1961) resulting in the (at first sight) paradoxical development of post-transfusion alkalosis (Litwin and Moore 1959; Howland et al., 1963).

In spite of the above considerations, the initial acid load of A.C.D. transfusions might clearly create cardiac difficulties if added to pre-existing acidosis. This would particularly be the case during inadequate total body perfusion or in patients who have some defect in citrate metabolism. It seems important, therefore, to assess the effects of the acids present in A.C.D. blood on cardiac function.

Calcium chloride added to the coronary perfusates had been shown to be capable of producing "heparin equivalence" even without correc-:tion of the acidosis normally present in A.C.D. blood. The next series of experiments was designed to show whether the same "heparin equivalence" could be achieved, at a lower calcium addition, if the acidosis was corrected prior to perfusion.

Method

Coronary perfusions were carried out in dogs using the preparation previously described. In each experimental preparation a dose of calcium was determined which, when added to the A.C.D. blood perfusates, gave changes in V.C.F. at the mid and end points of injection which indicated inadequacy of recalcification. Two groups of paired perfusions were then undertaken to dissociate correction of metabolic acidosis from respiratory acidosis.

Group A.

Ten pairs of perfusions were matched for calcium addition and PCO_2 and the effects of restoring the blood base excess to or near normality by the addition of 15-30 mEqu per litre of 8.4% sodium bicarbonate solution in one perfusion of each pair was assessed by 60-second coronary perfusions at 37°C as in the calcium experiments. Changes in V.C.F., T-waves, Q-T intervals and pulse rates were recorded (Table 2).

Group B.

Eleven pairs of perfusions were matched for calcium addition and base excess, and PCO_2 was reduced from the high figure normally found in

in A.C.D. blood (> 150 mm. Hg.) to normal or even subnormal levels in one perfusion of each pair. Reduction of PCO_2 was achieved by equilibration with air or oxygen of A.C.D. blood samples in a glass chamber rotating in a water bath at 37°C.

The effects on V.C.F. and E.C.G. were assessed by 60 second coronary perfusions at 37°C in a similar manner to those previously described.

Group C.

A further series of seven perfusions in which alterations of both respiratory and metabolic components of the acidosis in A.C.D. blood were made, was compared with a corresponding series of perfusions without such correction. In these sodium bicarbonate solutions (8.4%) was added to correct the matabolic acidosis before equilibration of the blood sample with air or oxygen in the 37°C water bath.

The results of the perfusions in Groups A, B and C are listed in Table 2, and summarised graphically in Figs. 20, 21 and 22 respectively.

Results and Conclusions

Group A.

Correction of the metabolic component of the acidosis of A.C.D. blood in one of each matched pair of perfusions of Group A resulted in an increase in ventricular contractile force at the end of sixty seconds of coronary arterial perfusion in nine of eleven tests (Fig. 20). There was no change in one (Perfusion 2 (2)) and a decrease in one (Perfusion 11 (4)).

The reason for the anomalous result in perfusion 11 (4) is not clear. The dog was moderately hypotensive during all its perfusions but there

there was no haemodynamic or biochemical difference, at the time of the perfusion in question, to account for the unusually large depression of contractility seen.

The average increase in V.C.F. in the remaining nine acid-corrected perfusions was +15%.

The only consistent change in the electrocardiograms was a tiny increase in the Q-T interval (approximately 1 mm. on the scale used, which equals 0.04 sec.).

Group B.

Correction of the respiratory component of the acidosis of A.C.D. blood in one of each matched pairs of perfusions of Group B (Fig. 21) resulted in an increase in ventricular contractile force in nine of the eleven tests. There was an insignificant decrease in two (Perfusions 6 (4) and 11 (5)).

It will be noted that the anomalous results were again in moderately hypotensive preparations. The average change in V.C.F. in the whole group was +31.5 per cent, a clear indication of the powerful cardiac depressive action of hypercapnia.

The electrocardiographic change characteristic of Group B was shortening of the Q-T interval, on average by 0.05 seconds. In no case was the Q-T interval lengthened. In this respect correction of hypercapnia resembled the action of calcium.

Group C.

The surprising conclusion from the data in Group C (Fig. 22) was

was that the effects of correcting both metabolic and respiratory aspects of the acidosis were not additive. The average increase in V.C.F. in this group was only +14 per cent - closely comparable with the figure obtained in Group A.

The T-wave and Q-T intervals showed no consistent pattern of change. This is perhaps not surprising since any alterations seen in Groups A and B took place in opposite directions.

Pulse rates at the end of perfusion were neither consistently speeded nor slowed by the manipulations of the acidosis in the perfusates of any of the three groups.

Production of metabolic alkalosis alone, without the addition of calcium, did not restore contractile force to a heart perfused with A.C.D. blood (See perfusion 4 (10)). Similarly metabolic alkalosis (Base excess +30) was used in two further perfusions containing calcium (5 (7) and 4 (7)) with increases of V.C.F. compared with control perfusion (5 (3) and 4 (3) respectively). These increases of +40% and +8% are insufficient to postulate any advantage for such gross over-corrections of acidosis in A.C.D. blood.

Discussion

Since infusion or perfusion of blood into the intact animal allows rapid elimination of any excess of carbon dioxide from the blood via the lungs, the changes in V.C.F. due to alteration of PCO_2 are of little clinical importance - though interesting as an isolated finding on cardiac physiology.

The improvements in cardiac contractility associated with correction of metabolic acidosis are relevant though somewhat disappointing in their magnitude. It may be that sodium bicarbonate solution 8.4% B.P., when it is given intravenously to hypotensive, acidotic patients, has both peripheral circulatory and central cardiac actions, which in combination result in the clinical improvements often seen. Only the central effects were investigated here. An average increase in ven-:tricular contractile force of 15 per cent occurred in 9 out of 10 experiments. This increase, though worthwhile in a myocardium critically depressed by acidosis, is minute compared with the cardiotonic effect of full dosage with calcium.

These findings are somewhat at variance with those of Baue and his colleagues (Baue et al., 1961) who found that, in the isolated dog heart, correction of acidosis in heparinised A.C.D. blood containing 0.5 grams calcium chloride per 500 ml. resulted in large increments in left ven-:tricular pressure. The organic buffer THAM was used, however, after initial studies had shown ".... bicarbonate buffer systems to be unsuitable".

The fact that THAM is able to cross the cellular membrane and, therefore, to correct intracellular acidosis may account for the differences in degree noted between the effects of THAM and sodium bicarbonate on myocardial contractility.

THAM (0.3 M solution) has in fact been used for the buffering of A.C.D. blood, converted for use in clinical open-heart surgery by the addition of heparin and calcium in the doses previously described by

ourselves (Foote et al., 1961). Urinary output was markedly increased in the first twenty-four post operative hours but no haemodynamic advantages were claimed (Nahas et al., 1964).

It is clear that correction of acidosis alone in A.C.D. blood is insufficient to restore myocardial contractility to normal. Used, as in the present experiments, for direct coronary perfusion, A.C.D. blood requires calcium or a combination of calcium and acid-base correction to restore V.C.F. to normal.

THE EFFECTS OF HYPERKALAEMIA ON MYOCARDIAL CONTRACTILITY

When red cells are stored in the cold, the potassium content of the red cells decreases and that of the plasma increases. Normally red cells contain about 100 mEqu. of potassium per litre of cells. After ten days' storage in A.C.D. solution, the plasma potassium is about 15 mEqu/l, after fourteen days' it is about 20 mEq/l (LeVeen et al., 1959; Moore 1959; Nealon 1960) and after twenty-eight days' about 30 mEqu./l. (Loutit et al., 1943).

High concentrations of potassium in the fluid perfusing the heart can produce arrest or ventricular fibrillation preceded by electrocardiographic abnormalities (Stewart et al., 1948; Sodi Pallares 1956; Winkler et al., 1938), Reynolds et al., 1951), and minor arrhythmias. This was known since the classical experiments of Ringer (1883). However, a high plasma potassium concentration does not happen easily during potassium infusions, owing to the very rapid equilibration of the ion into the extracellular spaces and into the cells themselves.

It has been shown, for example, that intravenous infusion of 0.6 mEqu. of potassium per minute in a 70 Kg. man had no effect on electrocardiogram or blood pressure. The experimental danger level of sustained potassium administration was 0.026 mEqu./Kg./minute (Corsa et al., 1950). This would be seen if IG of potassium chloride were given in 7.5 minutes intravenously to a 70 Kg. man. At slower rates of infusion it has been

been found that changes of less than 100 mEqu. in bK (total body pottasium) have small effects per se on serum potassium in adults (Burnell et al., 1956; Scribner and Burnell, 1956). By oral adminis-:tration in man, it was difficult to raise the plasma potassium concen-:tration by more than 3 mEqu./l., even when renal damage was present (Winkler et al., 1938).

Experimental infusion of isotonic potassium chloride solution (1.12%) in 5 dogs has been used to correlate electrocardiographic changes with plasma potassium levels (Winkler et al., 1938). A sequence of events progressing from increased T-wave amplitude at a mean of 6.6 mEqu/L. to disappearance of the P-wave and intraventricular block at 10.3 - 10.6 mEqu/L. and finally cardiac arrest at 14.3 - 15.8 mEqu/L. was described. A normal rate continued right up till the moment of arrest. The data were somewhat inadequate but suggested that there is a critical blood level of potassium which is lethal, and rate of injection is only important in so far as this level is reached. It should be noted that the infusion used had a potassium concentration of 150 m.Equ/L. (isotonic K Cl) and that plasma potassium values were obtained from peripheral venous blood which may not truly reflect the instantaneous levels reached in the blood perfusing the coronary arteries.

The combination of citrate and potassium was tested in three dogs by LeVeen and his co-workers (1959) and found to be lethal at a potassium dose of 2.5 mEqu/Kg. compared with 3.8 mEqu/Kg. using potassium alone.

Citrate alone produced only minor E.C.G. alterations at the dose rate tested and was not fatal in any dog. This was suggestive though not conclusive evidence of the synergism of hyperkalaemia and citrate intox-:ication in the dog.

Evidence of this synergism in man is lacking. There is an understand-:able reluctance to take frequent arterial blood samples in shocked patients during resuscitation and the potassium concentration of the transfused blood is seldom known. Hence the potassium concentration of the blood perfusing the coronary arteries in shocked states has seldom been clinically recorded and it is well recognised that potassium concentration in peripheral venous samples may bear little relationship to the potassium level in the heart in these circumstances.

It is to be expected, that if dangerous potassium cardiotoxic effects occur with clinical A.C.D. blood administration they will be profound in infants undergoing exchange transfusion. Miller, McCoord et al., (1954) found that, while blood stored for lessthan one week was innocuous, older blood stored for 9-21 days with a mean plasma potassium of 15 mEqu./L, led to serum potassium concentrations of 8 mEqu./L on two occasions after exchange transfusions. No E.C.G. changes were observed and no ill effects were reported.

A very rapid agonal rise in serum potassium of central heart blood occurs immediately after respiratory arrest (Winkler and Hoff, 1947). It has been speculated that this rise in plasma potassium is due to

to release of potassium from the liver, and that it is the cause of some instances of cardiac arrest during haemorrhagic shock (LeVeen et al., 1959). When plasma potassium concentration is found to be raised after or just before death, however, it is probable that the rise in potassium is a consequence, rather than the cause of the collapse.

A dynamic inter-relationship exists between different ions present in the plasma. Except in an artificial experimental way, it is impossible for one electrolyte in an intact animal to vary without being accompanied by synchronous alterations in all other electrolytes present in the body. It is possible to calculate the relation between the diffusible ions according to the Donnan Distribution Law (Harkins and Hastings, 1931). For example, experimental correction of acidosis was found to result in a decrease in serum potassium of 0.68 m.Equ./l per 0.1 pH units (Burnell et al., 1956). Curiously enough the increase in potassium was 0.50 mEqu./L. per 0.1 pH unit during correction of alkalosis. Similarly Howland notes that a decrease in pH of 0.014 units leads to an increase in ionised calcium of 0.05 mM/l. (Howland et al., 1957). In an extensive and striking series of experiments attempting to assess the effects of electrolyte changes on the E.C.G. in dogs, Roberts encountered this problem (Roberts and Magida, 1953). Intravenous infusions of potassium chloride, lithium chloride, ammonium chloride, hydrochloric acid, and sulphuric acid in the intact anaesthetised dog all produced strikingly similar E.C.G. patterns - sharp peaked increased

increased T-waves in Lead I : depressed S-T segments and inverted T-waves in Lead II. With the electrolyte infusions there was a variable reduction in pH, bicarbonate and sodium. Plasma potassium increased significantly to greater than 6.3 mEqu./L. in all experiments except when hydrochloride acid was infused. Inhalation of 20 per cent carbon dioxide, reduced pH to 6.82, caused increases in plasma bicarbonate and sodium but not potassium and produced only very minor E.C.G. changes.

Facts like these make the analysis of cardiac effects of intravenous electrolytes difficult, and are the main justification of the use of the coronary perfusion technique in the experiments described in this thesis. The individual variation of electrolytes was then under more simple control.

The Na/K ratio has been shown to be an important factor influencing cardiac contractility in its own right by the elegant experiments of Roebuck and his co-workers (Roebuck et al., 1962). In dogs subjected to hypercapnoeic acidosis for four hours and then hyperventilated with 100% oxygen (a process which, untreated, invariably leads to ventricular fibrillation, hyperkalaemia (Av. 9 mEqu./L.), and hyponatremia (Av. 125 mEqu./l)), it was proven that administration of hypertonic sodium chloride solution (1.5M) could maintain cardiac contractibility and lead to a decrease in plasma potassium. This was not an effect of hypertonicity since hypertonic mannitol-glucose solution did not protect the heart or alter plasma potassium. Correction of the acidosis with THAM

THAM (2 amino-2 hydroxymethyl 1, 3 propanediol) instead of by hyper-:ventilation with oxygen resulted in asystole rather than ventricular fibrillation. Injection of 1.5 molar sodium chloride solution at asystole led to resumption of cardiac function. The amount of sodium required to restore systole was ten times the amount of calcium used in a similar preparation. (Epstein et al., 1961; Roebuck et al., 1962). These works related plasma concentrations of potassium, sodium and calcium to cardiac function only on a basis of recordings of blood pressures and E.C.G. but are amongst the few which attempt correlation of electrolyte and acid-base changes and show in addition the effect on the intact animal of altering both metabolic and respiratory components of an acidotic state.

Besides ionic shifts due to transmembrane equilibration, the simpler matter of electrolyte mixing in the blood stream deserves mention. When blood or simple electrolyte solutions, with ionic concentrations ex-:ceeding the normal values, are infused intravenously the differences in ionic concentrations from those of the recipient animal are firstly minimised by admixture of the infusate with the rest of the venous return reaching the right side of the heart. Passage through the pulmonary capillary bed allows a small amount of equilibration with the pulmonary cells and intercellular space fluid. This blood then perfuses the coronary arteries when it is ejected from the left ventricle. Very rapid injection may interfere with this equilibration process so much that

that a bolus of infusate may conceivably reach the coronary ostia virtually undiluted by recipient blood. Clearly also the smaller the recipient's cardiac output the slower is his cardiac venous return and hence the more nearly will the right heart, pulmonary, and finally coronary artery blood resemble an intravenous infusate in ionic content. Intra-arterial or portal venous infusions on theother hand must pass through two capillary beds in the normal animal before reaching the coronary cirulcation. This allows more time for transcellular equilibration and gives a greater chance of complete mixture of an infusion with recipient blood. Only injections under high pressure at or very close to the coronary ostia are capable of causing an intra-arterial infusion to defy the torrent of blood pouring through the aortic valve with each systole, and enter the coronary circulation in significant quantity. This observation made by radiologists attempting coronary arteriography must serve to explain the findings that intra-arterial infusions of, for example, A.C.D. solution at the level of the coronary ostia did not produce cardio-toxic effects greater than those seen during intravenous administration (Nakasone et al., 1954).

In experiments involving intact animals, there is always the possibility that blood pressure changes are mediated by a primary effect on the vasculature. As early as 1938 Katz and Lindner showed that excess sodium produced temporary coronary arterial dilatation while concentrated potassium solutions gave long-lasting constriction at times almost to the

the point of complete coronary occlusion. The solutions used were completely unphysiological, however (K = 300 mgm%, for example) and these observations have no clinical significance. Physiological concentrations have been studied during perfusions of the vascular beds of the dog forelimb, kidney and heart (Haddy et al., 1963; Emanuel et al., 1959; Scott et al., 1961). Many ions were found to be locally vasoactive and this vasoactivity was mainly limited to the arterial side of the capillary. Elevation of the plasma potassium ion concentrations to levels less than twice the normal value produced arteriolar dilatation. Arteriolar dilatation also followed a local increase in hydrogen ion concentration, particularly in limb vessels. Calcium was the only ion which caused vasoconstriction when increased within ranges seen during life. Reduction of hydrogen ion also produced constriction (Fleishman et al., 1957).

Local excesses of acetate, pyruvate and citrate all caused vasodilatation while chloride, phosphate, sulphate and lactate seemed to be inactive.

In perfusion of the isolated heart with electrolyte solutions (deficient in proteins and oxygen-carrying red cells) the following observations were made, (Haddy et al., 1963). Hypokalaemia produced a dubious increase in ventricular contractile force as measured by a Brodie Bridge sutured to the left ventricle. Alkalosis (pH 8.36; bicarbonate 6.5 mEqu./l.) slightly increased the geometric resistance to flow through the coronary arteries. Hypercalcaemia of 6.4 mEqu./l. greatly increased contractile force and hypocalcaemia decreased it. Combination of hypokalaemia, alkalosis, hypercalcaemia and hypomagnesaemia enormously

increased contractile force, coronary perfusion pressure at fixed flow (76 ml/min.) and aortic pulse pressure above the values observed during a control infusion with normal electrolyte concentrations.

The effects of ions on the intact heart with whole blood perfusates were not studied because a satisfactory means of intermittent isolation of the coronary circuit had not been designed. With the circuit described in Figure 7 this difficulty has been overcome sufficiently to allow perfusions lasting 60 seconds at normal resting coronary blood flows, to be undertaken. The drawbacks to assessment of intra-:venous infusions mentioned above, were also obviated.

THE EFFECTS OF HYPERKALAEMIC CORONARY PERFUSION IN DOGS.

Method

The coronary perfusion circuit and procedure were as in the previous series. A potassium chloride solution containing 0.09 mEqu/ml. was used. 0 to 6.0 ml. of this solution were added to 60 ml. of either heparinised or converted A.C.D. blood warmed to 37°C. 50 ml. were retained for coronary perfusion, while the remainder was used for electrolyte and acid-base measurements. Calcium was added to A.C.D. blood in sufficient dosage to produce "heparin equivalence" in the dog preparation (0.6 - 0.7 ml. of 20 per cent calcium chloride solution B.P. per 50 ml. of A.C.D. blood). No alterations of acid-base status were combined with the potassium additions. The effects on V.C.F. and E.C.G. were analysed as before by comparing pairs of perfusions differing only in their potassium concentrations.

Results

A typical tracing showing the effect of a potassium concentration of 6.90 mEqu./l. in heparinised blood is shown in Fig. 16 (Perfusion 13 (7)). This particular perfusion showed that with a simple increase in potassium concentration, there was a change in V.C.F. of -50% at the end of 60 seconds of perfusion. The E.C.G. changes were not significant. The comparable perfusion of heparinised blood without the addition of

of potassium (Perfusion 13 (1)) gave a similar decrease in V.C.F. of -51% at the end of perfusion.

Comparisons were made between 12 pairs of perfusions which differed only by the addition of potassium. The 12 pairs are enumerated (using the nomenclature previously described) and the differences in V.C.F., E.C.G. parameters, and rates, are also listed in Tables 3 and 4. The experiments are arranged in ascending order of potassium addition to the test perfusates.

A.C.D. blood was employed in six paired experiments and heparinised blood in the other six. Figure 23 shows that when potassium concentration is increased in A.C.D. (closed circles) or heparinised blood (open circles) there is no systematic change in V.C.F. from that of a control perfusion. In six experiments there were increases in contractile force ranging from +62% to +1% relative to the respective control perfusions. Additions of potassium of 2.1 to 8.45 m.Equ./l. were made to the perfusates. In six experiments there were relative decreases in V.C.F. of -51% to -7% when similar additions of potassium were used (2.4 to 8.8 m.Equ./l). It is noteworthy that the four largest additions of potassium giving measured plasma potassium values of 11, 11.5, 11.9 and 14.45 m.Equ/l., produced differences from control V.C.F., at 60 seconds of perfusion, of +39%, +1%, -12% and -24% respectively none of which is a dramatic depression such as occurs when a grossly hypocalcaemic perfusion is com-:pared with its normocalcaemic (heparinised) control.

Ventricular fibrillation did not occur in any perfusion, even with a plasma potassium of 14.45 m.Equ/l. and indeed the electrocardiographic changes were remarkably slight at all levels of potassium addition. Elevations in T-waves were seen during four perfusions but in one of these the elevation was insignificant (0.5 mm). In the remaining eight perfusions minimal T-wave alterations or actual depressions of T-wave were seen. A considerable relative increase in pulse rate (63%) was seen after potassium addition in the first pair of perfusions but this was never repeated. Significant changes in Q-T interval were not seen.

No difference between recalcified A.C.D. blood and heparinised blood was detected.

D I S C U S S I O N.

It is unlikely that potassium concentrations higher than those used in the above experiments would reach the coronary arteries during clinical blood transfusion, because of admixture and dilution of the added blood with the recipient's own venous return. Sudden challenge of the myocardium with perfusates containing up to 14.45 m.Equ,/l. of potassium produced a very variable change in contractile force and minor electrocardiographic effects. Cardiac asytole or ventricular fibrillation did not occur.

Since the potassium content of stored A.C.D. blood remains below 15 m.Equ./l. for approximately ten days and since rapid equilibration of intravascular potassium takes place with the large total body potassium pool, the immediate danger of potassium arrest by stored blood administration, would not be suggested by these experiments. It should be remembered, however, that the A.C.D. blood perfusates used had had their citrate effects eliminated by the addition of calcium, and there remains the observation that hyperkalaemia is more cardiotoxic when it is combined with hypocalcaemia (LeVeen, et al., 1959) and that a solution of potassium citrate containing 1 mg. per ml. (approximately 10 mEq./l.) could produce cardiac arrest when injected into the root of the aorta during total body bypass (Melrose et al., 1955). Furthermore, the effects of more prolonged coronary perfusion with hyperkalaemic blood could not be assessed with the preparation described here.

In open-heart surgery, using cardio-pulmonary bypass, there may be a period at the onset of bypass when all the blood passing up the aorta to perfuse the myocardium is the priming blood from the machine. These experiments demonstrated the apparently innocuous nature of a coronary perfusion containing 11.0 m.Equ./l. of potassium (the highest concen-:tration at which a significant decrease in V.C.F. over control did not occur) when the calcium ion concentration was normal. It was con-:cluded that a large safety margin would be obtained by limiting the period of storage of A.C.D. blood intended for use as priming volume of a pump oxygenator, to three or four days when such levels of potassium concen-:tration are not attained.

THE EFFECTS OF HYPOTHERMIA ON MYOCARDIAL CONTRACTILITY

Introduction

There are claims that hypothermia carries a greater than normal susceptibility in the intact animal to the effects of citrate and acid infusions (Hara, M., et al., 1961; Blair, E., 1956; Brooks, D.K., 1965). Clearly the effects of hypothermia result from central cardiac actions and actions on the peripheral vasculature. No account is taken here of the peripheral effects. Only measurements of the changes in myocardial contractility arising from direct perfusion of the coronary vessels with cold blood will be discussed.

Method

The experimental preparation for coronary perfusion was as previously described. Perfusions were carried out in pairs using either heparinised blood or converted A.C.D. blood. Each cold (10°C) perfusion was paired with one at 37°C in which the perfusate was, as far as possible, comparable except in temperature. pH and PCO_2 values were corrected for temperature since all were measured by means of the Astrup micro-electrode method at 38°C. Changes in V.C.F. and E.C.G. from control period, were computed as in all similar experiments.

The temperatures of the perfusates were adjusted by immersion and rotation of the injection syringes, sealed by metal caps over their nozzles, in a water bath at 40°C or ice as appropriate. The actual temperature of the/

the blood was measured directly by means of a clean mercury thermometer inserted into the momentarily opened syringe, immediately prior to perfusion. With practice, it was usually necessary to carry out this manoeuvre only twice for each perfusate. The electrolyte content and acid-base status of each perfusate was checked.

It should be noted that, as far as acid-base status is concerned, only standard bicarbonate values are identical in each pair of perfusates since pH and PCO_2 are altered by changes of temperature.

RESULTS

Fifteen paired experiments were carried out and the results are given in Table 5. In five pairs heparinised blood was used and in ten pairs recalcified A.C.D. blood was employed. A representative hypothermic perfusion using "converted" ACD blood at 10°C is shown in Figure 17.

The general effect of hypothermic perfusion appears to be to cause an increase in V.C.F. though three minor decreases were seen. One reason for the lack of uniformity might be that cardiac temperature was lowered to different degrees in different dogs. No method of direct measurement of cardiac temperature, by means perhaps of a thermister probe, was available. However, if this explanation was correct the smaller dogs might be expected to be cooled below the critical failure temperature by virtue of their small heart size.

The results are, therefore, grouped according to weight of dog in Table 5 and it will be seen that there is no regular alteration of contractile force/

force as dog weight diminishes, though the greatest increase in force occurred in the largest dog (No. 13).

In 7 perfusions with cold A.C.D. blood the percentage change in V.C.F. after 60 seconds was positive compared with the corresponding percentage change in V.C.F. after 60 seconds of normothermic perfusion i.e. the heart beat was more forceful under conditions of hypothermic perfusion. It was very slightly less forceful on 3 occasions with A.C.D. blood. With heparinised blood all 5 hypothermic perfusions demonstrated increased V.C.F. compared with the corresponding normothermic perfusion. The mean increases in contractile force in the two groups were closely comparable viz., +40.2 for heparinised blood and +33.9 for A.C.D. blood.

DISCUSSION

It has been suggested that any increase in contractile force occurring during hypothermia could be due to increased diastolic filling, since slowing of ventricular rate is a constant feature of general body hypothermia. The truth of this hypothesis can be tested by assessing the effect of change of temperature on the empty beating heart. This has been done during total body bypass, using a Kay-Cross oxygenator and De-Bakey roller pump (Trede et al., 1961). During a period of total bypass the right ventricle could be seen to be collapsed and empty, though beating rapidly and vigourously, under normothermic conditions. The continuance of forceful contraction of the empty ventricle was confirmed by tracings obtained from a Brodie strain gauge arch sutured to the right ventricle. The heart rate decreased, the electrical cycle/

cycle became spread out on the E.C.G. tracing and the ventricular contractile force gradually increased, as oesophageal temperature decreased to around 20°C. In the majority of cases a change occurred at about this point and the contractile force of the heart showed an abrupt falling off, even if ventricular fibrillation did not supervene. ( ).

These experiments, though giving relevant information regarding the cardiac effects of cold, did not simulate the possibly dangerous effect of a bolus of very cold blood reaching the heart suddenly - as might happen during rapid transfusion of patients with banked A.C.D. blood taken straight from the 4°C refrigerator.

The coronary perfusion preparation previously described allows such an effect to be reproduced though it must be admitted that the preparation is still unphysiological in that the left ventricle does not pump blood against a resistance during perfusions. Owing to the presence of a left heart bypass during coronary perfusion the (pressure) work of the left ventricle is markedly reduced (Schenk, W.G., et al., 1964) and hence any tendency to sudden failure or fibrillation may also be diminished.

With these limitations in mind, the effects of sudden coronary arterial perfusion with blood at 10°C was assessed in the dog.

Hypothermia brought about by large transfusions has been blamed for some of the cardiovascular difficulties seen during such transfusions (Boyan and Howland, 1962, Boyan 1964). One case quoted by these authors had 35 units of cold A.C.D. blood during a six hour operation and developed cardiac arrest at 27.5°C. It will be realised that to lower the temperature of a patient weighing/

weighing about 50 Kg. by 10°C requires the loss of some 500,000 calories even if the patient were dead! 17,500 ml. of blood at 4°C could abstract only about 400,000 calories in having its temperature raised to 27.5°C. Even at a basal heat production of 1,200 Calories (Kilocalories) per day a patients' own heat production of 50,000 calories per hour could compensate for a large proportion of this heat loss. It seems likely that heat losses from large open wounds during prolonged operations are likely to have led to the severe degrees of hypothermia seen by these authors (Boyan and Howland). It is of interest, however, that in their plea for the warming of blood during massive rapid transfusion, thirty cardiac arrests occurred in 55 patients receiving cold blood transfusion while only eight out of 118 arrested with warmed blood. Furthermore, hypothermia did not develop in a patient receiving 32 units of warmed blood. E.C.G. changes of S-T segment prolongation, Q.R.S. distortion, ventricular extrasystoles and bradycardia - all of which are seen during citrate infusions at normothermia - were reported in hypothermic patients. One must accept, therefore, that the cooling of patients below 28°C by whatever means is hazardous, and should be treated or prevented.

On the basis of the experiments reported here, the ill effects of hypo-:thermic blood transfusion are not due to direct cardiac inhibition by cold. The sudden arrival of cold blood at 10°C did not produce gross cardiac arrhythmas or dramatic immediate changes in V.C.F. either with heparinised or "converted" citrated blood. This gives considerable reassurance that cold in itself is unlikely to do harm to the heart. Unmodified A.C.D. blood may reach the hypotensive, hypothermic myocardium in clinical transfusion. Since un-recalcified/

unrecalcified A.C.D. blood has such a catastrophic effect on the coronary perfusion preparation (e.g. perfusions 3 (7); 4 (10); 6 (3)); no attempt was made in the present group of experiments to assess the effects of perfusion of the heart with unmodified cold A.C.D. blood. Excessive sensitivity of the hypothermic myocardium to the effects of acidity and citrate, therefore, cannot be entirely ruled out as a potential source of cardiac dysfunction during A.C.D. blood transfusion. In the context of this investigation, however, which evaluates "converted" A.C.D. blood for priming an extracorporeal circuit, no contraindication to its use for hypothermic perfusion of the heart has been found.

One should remain alert to the possibility of the development of general hypothermia, during prolonged anaesthesia and operations, and massive cold blood transfusion, as a factor which may help to precipitate cardiac dysfunction so that steps to maintain normal temperature may be taken early. It would seem justified also to conclude that the intravenous administration of calcium salts, at the same time as hypothermic A.C.D. blood transfusion, should provide a factor of safety mitigating any detrimental effects of citrate on a heart possibly sensetised to citrate by cold.

CHAPTER 3.

An assessment of the feasibility and safety of using "Converted" A.C.D. blood for cardio-pulmonary bypass in dogs.

THE EFFECT OF CALCIUM ON ANTICOAGULATION OF BLOOD BY HEPARIN.

Heparin or a heparin-like substance is the naturally occurring anticoagulant in blood. It is a mucoitin polysulphuric acid ester found in greatest abundance in the mast cells particularly in the liver, (Gollmann, 1957). It is the only non-toxic anticoagulant which can be rapidly neutralised and, therefore, lends itself admirably to procedures requiring anticoagulation of a high order to cover a specific surgical or mechanical event. Heparin is the natural choice, therefore, as the anticoagulant in open-heart surgery using extracorporeal circulation.

During extracorporeal circulation heparin is gradually metabolised and excreted (Thomson and Ehrlich, 1964) at a rate varying with the individual patient, the duration of perfusion and the temperature of perfusion. At the end of the operative procedure residual heparin can be counteracted by giving an intravenous dose of polybrene (hexadi-:methrine bromide - Abbott), or protamine sulphate. This dose can be most accurately adjusted to requirement in an individual case by per-:forming a protamine (Perkins et al., 1956) or polybrene (Thomson and Ehrlich 1964) titration test. The latter test, assesses coagulation of heparinised blood by a series of polybrene dilutions. The level of heparin present in a 1 ml. sample of blood can be determined assuming a 1 mg:100 units ratio of polybrene : heparin equivalence. Polybrene is preferred by many for this purpose since it is much more stable in solution than protamine and, therefore, more reliable. The test is somewhat cumbersome since it requires a large volume of blood if the actual heparin level is not fairly accurately known beforehand.

An alternative is to measure the whole blood clotting time after addition of a known quantity of polybrene to 1 ml. aliquots of heparinised blood. The effect of additions of electrolyte solutions to heparinised blood can be assessed, using variation of "polybrene clotting time" as an index.

Since large volumes of citrated blood containing unusually high calcium concentrations were to be used in extracorporeal circulation, it seemed necessary to check whether calcium is liable to affect heparin metabolism in any way. That such might be the case had been suggested (Geszti O., et al., 1958). These authors purported to show a delayed anti-heparin action of intravenous calcium. Preliminary work by ourselves in vitro (Foote, Trede and Maloney, 1961), had demonstrated that the addition of 10% calcium chloride solution U.S.P. to heparinised blood was likely to precipitate premature coagulation in it, or at least to diminish its protamine titration value. Pre-:mature coagulation and reduction of protamine titration value were not seen in A.C.D. blood, converted for use in a pump-oxygenator circuit by the addition of 2,500 units heparin per 500 ml. and up to 9 ml. of 10% calcium chloride solution U.S.P. However, it seems desirable to investigate these phenomena more completely and to present some data demonstrating the safety of adding large quantities of calcium to a patient dependent for his safety on heparin anti-coagulation.

IN VITRO

Methods

Fresh heparinised human blood containing 2,000 I.U. per litre, and fresh to four-day-old A.C.D. blood were made available by the blood transfusion service of the Aberdeen Royal Infirmary. The blood came from people who, by reason of infectious jaundice, or other systemic disease, would otherwise have been rejected as donors. None of the donors had a known coagulation defect.

Heparin (1,000 units per ml.) B.P., Calcium Chloride 20% B.P. (diluted to a 2% solution for convenience in pipetting), sodium bicarbonate 8.4%, Sodium Chloride 2% and normal saline, solutions were added by means of fine graduated pipettes to blood, as dictated by the experimental protocols.

Polybrene dilutions were prepared containing 10 to 100 micrograms in 10 μG steps of polybrene per 0.1 ml. - the amount to be used in each test tube.

The experimental procedure, with minor variations was as follows. 1 ml. of heparinised or A.C.D. blood (usually now also heparinised) and otherwise modified as required, was added to each of a row of Lee-White coagulation tubes in a rack. To these tubes varying quantities of calcium chloride solution were added and mixed. The polybrene dilution selected for the test was now added and mixed. Thereafter each tube was tilted every half minute until firm coagulation, allowing to

total inversion of the tube, had occurred. This time was immediately noted. With practice, consistency in reading the clotting times was achieved, so that duplicate tubes seldom differed by more than one minute. Duplicate tubes could not be used routinely because of the limit to the number of tubes which one could comfortably pipette, read and record simultaneously.

All experiments were carried out at room temperature (approximately 21°C) and were timed by means of a stop watch. Careful note was made of the times of addition of the various solutions to blood, as well as the point at which coagulation occurred. Wherever, possible, aliquots of blood which were to be compared, were prepared in bulk, (e.g. 100 ml.) in order to minimise variations due to pipetting errors.

A. HEPARINISED BLOOD

Heparin was added in some experiments to give blood with a range of 2 to 5 units heparin per ml. for assay. The first part of each experiment consisted of a straight-forward polybrene titration of 1 ml. aliquots of heparinised blood against the serial dilutions of polybrene. The lowest polybrene concentration producing coagulation in less than 15 minutes, was selected for subsequent measurements of the coagulation times after additions of solutions of calcium chloride, sodium chloride 2%, or sodium bicarbonate 8.4%.

The effect of calcium chloride

In 6 experiments calcium was added in various doses to heparinised

heparinised blood prior to the addition of polybrene. The effect of delaying the polybrene clotting time estimation by up to twenty-four hours after the addition of calcium was assayed in all experiments (experiments 1 to 6 in Table 6).

The effects of hypertonic saline

During the course of these experiments, obvious differences between the control blood samples and those to which calcium chloride solution had been added became apparent, particularly when the calcium was in contact with the blood for 1-24 hours. It was possible that the phenom-:ena observed might have been due to the hypertonicity or high chloride content of the calcium chloride solution. Paired experiments with sodium chloride solution 2%, substituted for calcium chloride solution 2%, in one of each pair of experiments, served to eliminate these factors as explanations of premature coagulation in heparinised blood (experiments 7-10 in Table 7).

The effect of Acid-Base change

In all perfusion work the influence of acid-base alterations assumes prominence. The effects of alkalosis of a degree likely to be seen in perfusion fluids were investigated in six experiments by means of this coagulation system (experiments 11-16 in Table 8).

B. A.C.D. BLOOD

B. A.C.D. BLOOD

For each bottle of A.C.D. blood the minimum quantity of calcium required to produce coagulation is less than 30 minutes was found by titration of 1 ml. aliquots of blood against serial increments of calcium chloride solution 2%. The quantity required was usually .08 to .10 ml. of calcium chloride solution per 1 ml. of A.C.D. blood.

Heparin solution (2.0 to 5.0 units per ml.) was added and thereafter increasing quantities of calcium chloride solution (2%) were added as in the experiments using fresh heparinised blood. Quantities of calcium less than the pre-determined minimum to cause coagulation in the absence of heparin were omitted.

The effect of delaying the polybrene clotting time estimation by up to twenty-four hours after the addition of calcium was assayed in all experiments.

A. HEPARINISED BLOOD

Results

In Table 6 the horizontal rows of figures for polybrene clotting times show the changes produced by adding 0 to 0.4 ml. of 2% calcium chloride to 1 ml. aliquots of heparinised blood prior to the addition of polybrene.

Where there was no delay between the addition of calcium and poly-:brene (top row in each experiment) there is a consistent pattern whereby minor increases occurred in the clotting time until 0.2 ml. of calcium chloride was added. Thereafter rapid increases in clotting time attended the higher concentrations of calcium chloride.

Delay between calcium addition and polybrene clotting time estimation can be analysed by study of the vertical columns under the various calcium concentrations in Table 6.

When no calcium was added it was usual to find that, after lying out for 24 hours on the bench at 21°C, heparinised blood has a somewhat longer polybrene coagulation time than when it was fresh. Only in experiment 5 was the 24 hour coagulation time shorter than at the start of the experi-:ment. Shortening of polybrene clotting time, when assayed with a delay of 20-60 minutes after adding calcium, was consistently seen when the level of calcium chloride solution was 0.08 ml. per 1 ml. blood and greater. When the calcium to polybrene delay was 24 hours it was frequently found that at the higher calcium concentrations the tubes had

had coagulated spontaneously prior to the addition of any polybrene. (Recorded as a polybrene clotting time of 0 minutes: if observed before the end of the 24 hour delay period it is annotated accordingly). This phenomenon was observed in all experiments where the heparin concentration was 2 units per ml. and the calcium chloride was 0.1 ml. or more, but was not observed in any of the experiments where 5 units of heparin per ml. had been added. Substantial reductions in polybrene clotting time were observed in all tests employing high calcium dosage (0.1 - 0.4 ml./ml. blood) and a delay of 1 to 24 hours between additions of calcium chloride and polybrene. Coagulation of heparinised blood without the addition of polybrene or protamine was a disturbing discovery.

Table 7 compares, in four pairs of experiments, hypertonic calcium chloride solution (2%) with hypertonic sodium chloride (2%) solution. In view of the previous experiments a delay of 24 hours was employed in order to magnify any differences in coagulation. Coagulation without polybrene occurred in experiment 7 after the addition of 0.08 ml. calcium chloride solution per/ml. of blood. On the other hand polybrene clotting time increased with increasing concentrations of sodium chloride. A similar pattern was repeated in experiments 8 and 9.

At the higher heparin levels of experiment 10 a minor reduction of polybrene clotting time was seen at all levels of calcium addition but no coagulation without polybrene occurred. A minor increase in clotting time attended the addition of 0.4 ml. of 2% sodium chloride solution per

per 1 ml. of blood.

The results of varying the acid-base status of heparinised blood are given in Table 8 (Experiments 11 to 16). In each experiment three levels of base excess in the blood were produced by the addition of 8.4% sodium bicarbonate solution and the exact value determined by means of the Astrup micro-electrode technique. When no calcium was added (first vertical column of polybrene clotting time) no significant pattern of change with increasing base excess was seen. The familiar increases in clotting time at high calcium concentrations, when there was no calcium - polybrene delay, were again seen in experiment 11 (all levels of base excess) and experiment 12 (base excess zero only).

B. A.C.D. BLOOD

The results in a selection of typical experiments using A.C.D. blood are given in Table 9 (Experiments 17-20). Coagulation occurred in less than 30 minutes in unheparinised A.C.D. blood when 0.1 ml. or more of 2% calcium chloride solution was added to 1 ml. of blood (Experiment 17). In two of six other blood samples 0.08 ml. of calcium chloride were sufficient.

As the time of contact between the added calcium and the blood increased, before polybrene assay, a diminution of polybrene clotting time was seen in every case.

When the time of contact ($CaCl_2$ to Polybrene Delay in Table 9) was 24 hours, premature coagulation of the blood occurred where the initial heparin level was 2 units per ml. Only slight shortening of polybrene clotting time occurred with the higher heparin dosage of 5 units per ml. (c.f. experiments 1 to 6).

Identical findings were made in 4 further experiments which are not, therefore, reported in detail.

Discussion and conclusions

The diminutions in polybrene clotting times seen after calcium additions to both heparinised blood and heparinised A.C.D. blood may be manifestations of an antiheparin effect of calcium. On the other hand clotting may merely be activated through a mechanism bypassing the antithrombin and antithromboplastin actions of heparin.

Certainly when calcium chloride 2% solution addition was 0.08 ml. per ml. of blood (or greater) there was an invariable tendency for polybrene

polybrene clotting time to diminish with the passage of time after calcium addition. This diminution led to the coagulation of hepar-:inised blood containing 2 units of heparin per ml., in less than twenty four hours. When heparin level was increased to 5 units per ml. no instances of spontaneous coagulation without the addition of polybrene were noted in either heparinised or A.C.D. blood.

The essential point of these experiments, therefore, is that if one wishes to avoid the possibility of premature coagulation in heparinised blood when calcium is added, either 1) the heparin dosage must be 5 units per ml. (5,000 units per litre) and/or 2) the calcium dosage must be less than 0,08 ml. of 2% calcium chloride B.P. per ml. (8 ml. of 20% calcium chloride solution B.P. per litre).

IN VIVO CLOTTING

Since in vitro testing had suggested some degree of heparin antag-:onism by calcium, it was relevant to examine the *in vivo* action of calcium on anticoagulation by heparin. No comparable study was found in a search of the literature apart from the previously quoted work from Peking (Geszti O., *et al.*, 1958).

Method

In a series of experiments four dogs weighing 9.3 to 16.4 Kg. were anaesthetised with Pentobarbital and given intravenous injections of 300 units heparin per Kilogram body weight. The disappearance of heparin from the circulation was assayed at fifteen minute intervals thereafter, for two hours, by recording the polybrene coagulation times in blood withdrawn into Lee-White coagulation tubes at the appropriate inter-:vals. In most experiments (Dogs 1-3) thrombin times were estimated on plasma obtained at the same time intervals. All polybrene coagulation studies were started immediately after withdrawal of the blood samples from the indwelling intravenous cannulae. For the thrombin time studies the plasma was separated immediately, but the tests were not done till the end of the polybrene experiments - usually a matter of 20-30 minutes after the last blood sample was withdrawn. Care was taken to discard the blood in the lumen of the cannula before taking the samples for coagulation studies.

Each dog was used as his own control. The disappearance of an initial

initial dose of heparin after intravenous injection of one or more doses of calcium chloride (20% calcium chloride B.P.) was assayed in a similar manner in further experiments, and the results compared with the control injection of heparin. The results for the titrations against 10μ of polybrene are given in Table 10. The thrombin times are shown in Table 11. Calcium injections are designated by the letter "C" under the coagulation time for the blood sample which immediately preceded the injection.

Dog I had 3 heparin assays in addition to its control. In two experiments equal doses of 0.2 ml./Kg. of calcium chloride 2% solution B.P. were given 30, 45 and 60 minutes after the heparin administration. These experiments can, therefore, be compared with each other, as well as with the control, and give an indication of the degree of reproducibility of the experiment in the same animal.

In the third calcium experiment in Dog I and in one experiment each on Dogs II and III equal doses of 0.3 ml/Kg. of calcium chloride solution were injected 0, 15 and 30 minutes after the heparin.

In Dogs II and IV a single dose of 0.3 ml./Kg. of calcium chloride was given at time zero in one experiment each.

In Dog IV a steady continuous infusion of calcium chloride solution to a total of 2 ml./Kg. was given throughout one experimental period of 120 minutes.

A variety of total doses and timings of calcium administration were thus explored and the effects on heparin anti-coagulation analysed.

Results and Conclusions

The general conclusion from study of all the results recorded in Tables 10 and 11 is that no effect of calcium on heparin anticoagulation *in vivo* is discernable at the doses or time schedules investigated.

Comparisons between calcium experiments and their controls where heparin alone was given, reveal only minor and irregular differences no greater than can be accounted for by the variation from day to day with the identical protocol (Dog I). If calcium had a marked antiheparin action *in vivo* one would expect a major and consistent reduction in polybrene clotting time or thrombin time after calcium administration but this was not seen even as a result of the massive continuous infusion of 2ml./Kg. of calcium chloride solution in 120 minutes, in Dog IV.

Figure 24 demonstrates in graphic form the close similarity of the four heparin disappearance curves obtained in Dog I. Only the poly-:brene clotting times at 90 minutes after heparin injection show signif-:icant variation.

The doses of calcium used (0.2 - 0.3 ml., 20% calcium chloride solution B.P. per Kilogram of body weight) were less than those which would be employed in priming a Kay-Cross oxygenator with converted A.C.D. blood. However, the calcium was unopposed by the citrate of A.C.D. blood and ought, therefore, to have achieved maximum effect, particularly in Dog IV; experiment (1).

The/

The rapid diffusion of calcium out of the intravascular compartment and immediate reduction of calcium ion concentration by the plasma proteins may explain the lack of antiheparin action of calcium seen in these experiments. Whatever the explanation, these experiments give some reassurance that antagonism of heparin by calcium, is not of major importance *in vivo*.

EVALUATION OF CONVERTED A.C.D. BLOOD USED FOR EXPERIMENTAL TOTAL CARDIO-PULMONARY BYPASS

The demonstration that citrated blood could be made equivalent to heparinised blood, at least as far as its effects on cardiac contract-:ility and electrocardiograms were concerned, made the application of "converted" A.C.D. blood to extracorporeal circulation in cardiac surgery a logical next step. Here a large priming volume of blood in the oxygenator and associated tubing is suddenly exchanged into the patient's cardio-vascular system at the onset of cardio-pulmonary bypass. The priming volume of a Kay-Cross rotating disc oxygenator and circuit, for example, may be as large as four litres and this volume may be perfused into the recipient in one minute or even less. Quite clearly this rate of citrate load far exceeds the toxicity levels eluc-:idated by many workers (e.g. Adams et al., 1944; Watkins 1953; Nakasone et al., 1954; and Firt et al., 1957). At the onset of bypass a large proportion of the blood entering the coronary arteries (by retro-:grade flow up the aorta) is the blood used for priming the extracorporeal circuit. The danger of such coronary perfusion with A.C.D. blood, particularly in a patient suffering from a cardiac defect and the trauma of thoracotomy, need no further elaboration.

TOTAL CARDIAC BYPASS

Method

Thirteen mongrel dogs weighing 12.2 to 28 kilograms had light anaesthesia induced with intravenous sodium thiopental and a cuffed endotracheal tube was passed. Narcosis was maintained by intermittent doses of the same agent or by the introduction of halothane through an intermittent positive pressure respirator. Aseptic surgical technique was employed. Median sternotomy was performed with care to achieve complete haemostasis. The pericardium was opened. Heparin (1.7 mg/Kg.) was given intravenously before any cannulations were carried out. Both venae cavae and one femoral artery were cannulated with appropriate Bardic cannulae and the dogs were placed on cardio-pulmonary bypass using the Kay-Cross rotating disc oxygenator and De Bakey pump system illustrated in Figure 25.

The following parameters were monitored on an Offner 8-channel ink-writing recorder.

1. Aortic and central venous pressures by means of polyethylene catheters introduced via the femoral vessels and connected to Statham strain gauges.
2. The electrocardiogram Lead II.
3. The occipito-frontal electroencephalographic lead.
4. Ventricular contractile force (V.C.F.) by means of a Walton-Brodie Strain gauge arch sutured to the wall of the right ventricle in eleven experiments.

In dogs 7 to 10 of the series, whole blood pH, buffer base, plasma potassium, plasma sodium, plasma haemoglobin and haematocrit were determined, in the priming blood, and in the dogs immediately before perfusion, after 4 and 20 minutes of perfusion, and 1, 2 and 3 hours after perfusion. pH was measured with the Beckman Model H-2 pH meter, using a saline-filled blood-type glass reference electrode maintained at constant temperature in a water bath. Values were corrected, by means of the Rosenthal factor, to the body temperature indicated by an oesophageal thermister probe which was calibrated against a mercury thermometer (Rosenthal 1948). Buffer base was derived by the method described by Peirce (Peirce et al., 1959) which is similar to the Astrup method. Plasma potassium and sodium were determined with a flame spectro-photometer and plasma haemoglobin by the method of Flink and Watson (Flink and Watson 1942). The biochemical data from experiments 7 to 10 are set out in Table 13.

In three experiments the donor blood used was fresh heparinised blood. In the ten other experiments the priming volume was A.C.D. blood "converted" by the addition of heparin (2,500 units per 500 ml.) and calcium. A dosage of 9 ml. of 10% calcium chloride solution U.S.P. per 500 ml. A.C.D. blood was used in six experiments and 6 ml. of 10% calcium chloride solution U.S.P. per 500 ml. in the last four. In two experiments (Dogs 6 and 10) the V.C.F. was not monitored after electrical faults had occurred in the recorder. The citrated priming blood in all these experiments was less than 48 hours old though occasionally a

a bottle of older A.C.D. blood, up to 14 days old, was added during the post-perfusion phase.

Three per cent carbon dioxide was present in the oxygen supplied to the oxygenator during cardiac bypass. Flows of 53 to 121 ml. per kilogram per minute (mean 93 ml/Kg/min) were employed for a twenty minute period of perfusion. Longer perfusions were not thought necessary since the most testing point in differentiating from hep-:arinised blood perfusions, is the initial mixing and equilibration of the donor and recipient blood volumes, which ought to be complete in twenty minutes. Full flows were given right from the onset of perfusion without any period of partial bypass, in order that the test would be as stringent as possible.

At the end of perfusion the dogs were transfused to a satisfactory systolic blood pressure, rapidly decannulated and the incisions closed after the removal of the Brodie bridge and the insertion of pleural drains. Protamine (2 mg/Kg) was given slowly intravenously. The animals were closely observed for several hours and received additional transfusions of unconverted A.C.D. blood as indicated by external blood losses.

If the immediate post-operative period was survived, the dogs were sacrificed and autopsied from three to seven days later. Histo-:logical studies were made of heart, lung, liver, kidney and spleen.

The electrically recorded data on blood pressure, venous pressure, V.C.F. and E.C.G. were analysed and recorded in tabular form, in a

a manner similar to that described for the coronary artery perfusion studies.

Results

All dogs except one survived the operative procedure. Of the remainder ten lived and were apparently healthy when sacrificed three to seven days post-operatively. No gross or permanent neurological signs were noted. Apathy was marked in dogs 3 and 7, for the first 24-48 hours but rapidly resolved thereafter. Hind limb weakness occurred in most of the dogs for the first few days but was probably related to general weakness and bilateral femoral arterial ligations rather than to central nervous system damage. Reluctance to move the hind limbs was seen in two of the three dogs perfused with heparinised blood. One dog (Dog 7) was thought to have right external strabismus but this had disappeared by the evening of the day of operation.

The dogs which died were (1) Dog 1. After an apparently straightforward perfusion this dog was returned to its kennel. It was found dead the next morning : no gross lesion was found in the chest, abdomen or brain to account for death.

(2) Dog 5. This was a small animal which showed severe hypotension, electrical coupling and a grossly abnormal electrocardiogram prior even to cannulation of the venae cavae or administration of any citrated blood. It probably had a considerable overdose of halothane and its death shortly after bypass is believed not to be attributable either to per-:fusion or to A.C.D. blood.

(3) Dog 8. There was a thirteen minute period of superior vena caval occlusion due to buckling of the tip of the caval catheter. This was corrected and perfusion was able to proceed normally. The dog was returned to its cage in good alert condition but was found dead 24 hours after perfusion. Post-mortem examination revealed a large haemothorax.

The electrocardiograms showed no characteristic changes during perfusion in Dogs 7 to 13. As has been reported by others using heparinised donor blood, transient T-wave inversion was not uncommon during the recovery period (Darby et al., 1958). In three of the dogs in which the donor blood contained 9 ml. of 10% calcium chloride solution U.S.P. per 500 ml. A.C.D. blood, normal electrocardiograms persisted throughout perfusion. In Dogs 3 and 4 minor alterations of R-wave voltage and height of the T-wave were noted during the first few minutes of bypass. Reference has already been made to the bizarre E.C.G. patterns which existed in Dog 5 prior even to cannulation.

Table 12 gives the figures recorded for dog weight; average perfusion rate during 20 minutes of cardio-pulmonary bypass; amplitude (in millimetres) of the V.C.F. tracings from the Walton-Brodie strain gauge (pre-perfusion, immediately before starting perfusion, after 1, 3 5, 10, 15 and 20 minutes of perfusion, after decannulating the venae cavae, and finally just before removal of the gauge); and systolic blood pressure at three points during each experiment - pre-perfusion, post-perfusion and one hour post-perfusion. The percentage change

change in V.C.F. from that obtaining immediately before starting perfusion, was calculated from the data for each group of perfusions, and is recorded in Table 12. Only data from experiments 1-4 were used in the calculations for the group receiving A.C.D. blood containing 9 ml. of calcium chloride solution per 500 ml. since the anaesthesia in Dog 5 was technically faulty.

The changes in "V.C.F." are summarised graphically for the three groups of dogs in Figure 26. In this figure the behaviour of three dogs perfused with heparinised blood is compared with that in the two groups where "converted" A.C.D. blood was used. The increase in V.C.F. commonly seen in the early period of perfusion with A.C.D. blood also occurred when fresh heparinised blood was used to prime the extra-:corporeal circuit. Myocardial depression was not seen and, indeed, a "rebound" increase in V.C.F. frequently followed the end of the A.C.D. perfusions.

Perusal of the biochemical data reveals the following:

1. Initial pH (mean 7.40) decreased immediately to a mean of 7.13 with the addition of the highly acid A.C.D. blood (mean pH 7.02).

   By the end of perfusion, mean pH had risen to 7.26, and after a minor decrease immediately after operation, had increased to 7.28 three hours post-operatively.

2. These changes in pH were mirrored by concomitant changes in buffer base. The normal buffer base in these dogs, as determined

determined by our method, was about 40 mEqu.per litre - somewhat lower than in the human. Significant metabolic acidosis had disappeared by the third post-operative hour.

3. There was a small (0.1 to 0.7 mEqu. per litre) but constant decrease in plasma potassium concentration at the end of the period of bypass which can not be accounted for by dilution with A.C.D. blood. The average plasma potassium in the donor blood was in fact slightly greater (3.10 mEqu.per litre) than that of the recipients' (3.03 mEqu. per litre).

4. Plasma sodium remained constant throughout the period of obser-:vation.

5. Moderate haemodilution persisting into the post-operative period was evident from the decrease in recipient haematocrit (mean 52) to a mean value of 43 at the end of perfusion.

6. Haemolysis was moderate in two experiments, slight in one and severe (3,160 mgm. per 100 ml.) in one case (Dog 10). The reasons for this variation are not certain. No cross-matching of dog's blood was done and some degree of incompati-:bility may be the explanation. All four dogs survived the operative period and Dog 10 with the highest haemolysis figures was a long-term survivor.

Long-term survivors showed no histological abnormalities. In those dying acutely the major changes were those of atelectasis and early pneumonia.

DISCUSSION

These studies established that survival in dogs after total body perfusion with converted A.C.D. blood was not attended by any undue biochemical or cardiac functional disturbances. A moderate metabolic acidosis persisted into the post-operative phase but no active steps were taken to correct this with intravenous sodium bicarbonate. There was a strong tendency towards return to normality of acid-base status as might be expected from the knowledge that metabolism of citrate eventually yields bicarbonate. The only statistically significant ($P < 0.05$) change in plasma electrolytes was the transient slight decrease in plasma potassium at the end of perfusion which would be of no clinical importance.

The stage appeared set, therefore, for a clinical trial of the use of "converted" A.C.D. blood as priming volume for heart-lung machines. The choice of calcium dosage to be used for the conversion was the major decision to be made. Theory, coronary perfusion exper-:iments and whole body perfusions in dogs all concurred that 6 ml. of calcium chloride solution 10% U.S.P. (20% B.P.) gave the requisite calcium ion concentration in 500 ml. of heparinised A.C.D. blood for maintenance of normal contractility. Excess calcium should not be used since large increases in calcium ion concentration have been shown to be cardiotoxic (Hoff et al., 1939) particularly in the digitalised subject (Golden and Brams, 1938; Wall, 1939) - though the synergism of calcium and digitalis in clinical dosages had been denied on experimental grounds (Smith et al., 1939). Furthermore, complete

complete chemical neutralisation of citrate is not essential if the initial infusion rate of A.C.D. blood is slow enough for the normal mechanisms of the body (mobilisation of calcium from bone; citrate metabolism in liver, kidney and muscle) to deal with any residual excess of citrate in the infused blood. It was decided, therefore, to use 6 ml. of 10% calcium chloride solution U.S.P. to convert each bottle of A.C.D. blood after heparinisation, and to employ a period of partial cardiac bypass at the outset of perfusion in order to allow gradual mixing of the extra- and intra-corporeal blood volumes.

CHAPTER 4

The Use of "Converted" A.C.D. blood for Human Extracorporeal Circulation.

THE USE OF CONVERTED A.C.D. BLOOD FOR HUMAN EXTRACORPOREAL CIRCULATION.

The preceding experimental evidence had demonstrated the feasibility of using converted A.C.D. blood for total cardio-pulmonary bypass in dogs. No evidence was discovered which would indicate that heparinised blood is superior to converted A.C.D. blood as far as the effects on the cardio-vascular or blood coagulation systems are concerned. When heparin in adequate dosage (5,000 units per litre) was used to convert A.C.D. blood the presence of high concentrations of calcium did not induce premature coagulation of the blood. The clinical application of converted A.C.D. blood for extracorporeal circulation, therefore, appeared justified. The correction of the metabolic and respiratory acidosis normally present in A.C.D. stored blood, though possibly offering an initial, but evanescent advantage, was not thought to be essential as long as (1) the donor blood volume (priming the machine) was less than that of the recipient, (2) perfusion rates were adequate to ensure the normal rapid metabolism of citrate and accumulated inorganic acids in the priming blood, (3) the acid buffering capacity of the recipient was normal at the onset of cardio-pulmonary bypass and (4) the ability of the recipient to metabolise citrate, lactate, pyruvate etc. was essentially normal.

The first three of these criteria are under the control of the cardiac surgicical team. The fourth was thought to rule out, at least until further experience was obtained, the application of "converted" A.C.D. blood to the perfusion of patients suffering from cirrhosis of the liver,

liver, prolonged cardiac failure or serious renal disease, and also for hypothermic perfusions. Infants also were rejected because of the high priming volume of the extracorporeal circuit relative to the patients' blood volume, the inferior buffering capacity of the blood in small children, and doubts about their ability to handle a large sodium and metabolic acid load.

A clinical study was carried out at the U.C.L.A. Medical Center, Los Angeles, California in 1960 in the cardiovascular surgical service of Dr. William Longmire and Dr. J. V. Maloney, Jnr.

In that study an extensive biochemical and haematological comparison was made between ten patients undergoing operation for intracardiac defects using extracorporeal circulation in whom the priming volume of the Kay-Cross oxygenator was converted one-day-old A.C.D. blood and eight patients in whom heparinised blood, also drawn the previous day, was used. The patients chosen for the A.C.D. trial had relatively simple surgical defects unattended by pulmonary hypertension, cardiac failure or previous operation. Several of the patients of the heparinised blood series had more complicated congenital defects, so that the valid comparisons related only to biochemical and haematological data in the para-perfusion period, and not to operative procedures, duration of bypass, length of cardiac arrest or to ultimate survival.

A detailed account of the first A.C.D. perfusion is given below and in Table 14, since this was the first recorded occasion on which solely

solely A.C.D. blood was used in a large-prime, extracorporeal circuit in man.

CASE RECORD

The patient was a white adult male aged 33, weighing 63.0 Kg., whose main complaint was mild dyspnoea on exertion. Routine examinations revealed the typical systolic murmur of pulmonary stenosis. Systemic blood pressure was normal and there was no cyanosis, finger clubbing or sign of cardiac decompensation. He was not receiving digitalis. Chest X-Ray revealed gross right ventricular enlargement. Preliminary cardiac catheterisation studies showed no evidence of intra-cardiac shunts. There was a gradient of 30 mm. mercury between the right ventricle and pulmonary artery. Angiography suggested the presence of infundibular stenosis in addition to a well-marked pulmonary valvular stenosis.

On 16th March, 1960 anaesthesia was induced with sodium thiopentone and continued with Demerol, Nitrous oxide and oxygen using a Bird respirator. The chest was opened by a sternum-splitting approach. The venae cavae and right femoral artery were cannulated after the intravenous administration of 107 mgm. of Heparin.

A 23-inch Kay-Cross oxygenator and De-Bakey pump circuit were primed with 4,000 ml. of A.C.D. blood, drawn 18 hours prior to operation and "converted" by the addition of heparin and calcium. To each 500 ml. unit containing 120 ml. of A.C.D. solution B (USP) 2,500 units of heparin were well mixed with the blood before the addition of 6 ml. of 10 per cent

cent solution of calcium chloride U.S.P. The conversion and priming were carried out half-an-hour before perfusion and the blood was equilibrated with 3 per cent carbon dioxide in oxygen supplied to the oxygenator at 10 litres per minute. The blood temperature was maintained at approximately 35°C by a heating coil around the oxygenator.

An initial period of five minutes partial bypass to allow gentle mixing of the donor and recipient bloods was employed. Thereafter total cardio-pulmonary bypass was continued for a total perfusion lasting 28 minutes. A mean blood flow of 3,000 ml. per minute was maintained after the first three to four minutes of partial bypass.

Pulmonary valvulotomy and resection of the hypertrophic infundibular stenotic muscle were performed. Anoxic cardiac arrest lasting ten minutes during perfusion was followed by ventricular fibrillation. Defibrillation was achieved by electrical countershock. At the end of extracorporeal circulation, rapid decannulation was accomplished and circulating heparin was neutralised with protamine (155 mgm. in two doses). Normal blood coagulation returned and the chest was closed.

The post-operative course was completely uneventful. The patient left hospital on his eleventh post-operative day, entirely symptom free.

Monitored Data.

As with all subsequent patients in the series, the following measurements were made:-

1. Systemic blood pressure and central venous pressure by means of fine polythene cannulae inserted into the radial artery and superior

superior vena cava respectively, and connected via Statham strain gauges to a Sanborn direct writing two-channel recorder. Each channel was calibrated before and after perfusion against a mercury manometer.

2. The electrocardiogram (Lead II) and the occipito-frontal electroencephalogram were separately recorded by another direct-writing oscillograph.

3. Temperatures of patient and extracorporeal blood, by thermister probes in the mid-oesophagus and the Kay-Cross oxygenator end-plate respectively.

4. Plasma sodium, plasma potassium, by means of a flame spectrophotometer.

5. Plasma haemoglobin (Flink and Watson, 1942).

6. Arterial whole blood pH, PCO_2 and Standard bicarbonate by means of the Radiometer micro-electrode system (Astrup 1960) and the nomogram of Andersen and Engel (1960). Values of pH and PCO_2 were corrected to the temperatures of the blood at the time of withdrawal using the Rosenthal factor (Rosenthal 1948) and the line diagram of Bradley and his co-workers (Bradley et al., 1956) respectively.

7. Plasma chlorides (Kingsley and Dowdell, 1950).

8. Haematocrits, white cell counts, Rees-Ecker platelet counts and Lee-White clotting times were done by Standard clinical laboratory methods.

The various biochemical parameters were recorded in the patient pre-operatively; after induction of anaesthesia; in the oxygenator priming volume; after 2, 5, 10, 15 and 28 minutes of perfusion (end of bypass): 1, 2, 4 and 24 hours post-operatively, taking the end of bypass as the start of the post-operative period. The patient was followed up by daily haematocrit readings and measurements of fluid balance.

Table 14 summarises the biochemical data obtained from this first perfusion with"converted" A.C.D. blood.

Results

The haemodynamic behaviour of this patient followed exactly the normal pattern seen with patients and dogs undergoing extracorporeal circulation with heparinised blood. A smooth transition to partial bypass "in parallel" with the patients own circulation was achieved. No electro-cardiographic abnormalities appeared during the period of mixing of priming volume and the patient's own blood, nor did they occur when total bypass was started by tightening the tapes around the caval cannulae. Ventricular fibrillation ensued as a result of anoxic arrest of the heart by cross-clamping the aorta during the intra-cardiac procedure. Defibrillation by electrical countershock was easy after the right-ventriculotomy had been closed. Adequate cardiac output followed cessation of extracorporeal circulation and there was never any anxiety from this point of view.

The severe metabolic acidosis present in the priming blood (standard bicarbonate 7.3 mEqu./L.) equilibrated with the patient's own blood within two minutes on partial bypass and thereafter there was a steady decrease

decrease in metabolic acidosis during perfusion. A minor degree of acidosis (standard bicarbonate 17.4 mEqu/L) persisted 30 minutes after the end of bypass, associated with a pH_B of 7.64 due to over-ventilation by the anaesthetists. Thereafter all acid-base figures were within the normal range.

Changes in electrolyte concentrations and haematocrits were of a minor nature, though the potassium figures are noteworthy. A decrease in plasma potassium from 4.91 mEqu/l. in the priming blood and 4.13 mEqu/l. in the patients blood to 3.16 mEqu./L. at the end of bypass was noted: Rapid return to normal levels by the 4th post-perfusion hour occurred.

The most striking changes in the haematological data were in the white cell counts. Normal counts persisted throughout the period of bypass. One hour after cessation of perfusion the white count was 27,000 per cu.mm., remained high throughout the first day, and was still elevated to 11,000 per cu. mm. twenty-four hours post-operatively. A depression of platelet count to 74,400 per cu.mm. occurred twenty-four hours after perfusion, but returned to normal (160,000 per cu. mm.) in four days.

Haemolysis during perfusion was slight. The plasma haemoglobin at the end of perfusion was only 40.3 mgm. per 100 ml. and had returned to 0, five hours after perfusion.

No difficulties were encountered with heparin reversal. The Lee-White whole blood clotting returned to a normal figure of 6 minutes within 30 minutes of the administration of the second dose of protamine. Post-operative bleeding was minimal. The patients' haemoglobin level was well maintained throughout the rest of his stay in hospital. The haemato-:crit 7 days after operation (not quoted in the Table) was 41 .

DISCUSSION

There can be little argument with the fact that blood collected immediately before operation with heparin as the sole additive, is the most physiological medium for extracorporeal circulation. It has normal pH and electrolyte concentrations, fully viable erythrocytes and, presumably an intact quota of factors necessary for coagulation. Storage for longer than a few hours in any anticoagulant mixture, however, results in changes in each of these modalities to a degree varying with the actual process employed. On the combined counts of (a) post-transfusion red cell survival (b) rate of increase in plasma potassium (c) spontaneous haemolysis and (d) persistence of clotting factors, V, VII and antihaemo-:philic globulin, acid-citrate-dextrose (A.C.D.) solution is demonstrably superior to all other preservative mixtures in common use, especially if the blood is collected in plastic containers (Gibson 1959). The only agent claimed to give equivalent erythrocyte preservation during the first week of refrigeration storage at 2° to $6^{\circ}C$ is Edglugate -Mg. mixture (Smith et al., 1959). However, in common with blood stored in heparin-dextrose solution (Abbott et al., 1958; Lobpreis et al., 1960) alkaline-citrate mixtures (Loutit et al., 1943; Mollison and Young, 1942) and EDTA alone (MacGovern et al., 1959; Sprague et al., 1953) or decalcified by passage across a cation exchange column (Schechter, et al., 1962), rapidly mounting plasma potassium levels are seen in Edglugate -Mg. blood. Safe levels of potassium (less than 7 mEqu./L) are found for at least five days in A.C.D. blood. There is also some suggestion in the literature that clotting factors are less well preserved by all the above

above processes than by A.C.D. (MacGovern et al., 1959; Smith et al., 1959; Wurzel et al., 1959).

If absolutely fresh heparinised blood is not readily available or its procurement puts excessive strains on the blood banking facilities of a centre carrying out an open-heart surgery programme, then converted A.C.D. blood appears to offer an attractive alternative. The advantages are several. Citrated blood is universally available and if unused for a perfusion can, unlike heparinised blood, be returned to the blood bank. It is not unusual for up to twenty pints of blood to be withdrawn for high-prime extracorporeal circulation machines incorporating oxygenators of the Kay-Cross or Melrose types. If a last minute cancellation of a perfusion occurs, or if there is minimal operative blood loss, very large quantities of heparinised blood may have to be discarded.

Furthermore, cross-matching of A.C.D. blood can be done at leisure on blood withdrawn several days before operation with greater convenience to the donors. As pointed out previously, plasma potassium increases but slowly in A.C.D. blood, and clotting factors are reasonably well preserved. Cardiotoxic levels of potassium as judged by the results of coronary perfusion with hyperkalaemic blood, would not be remotely expected if a limit of five days of storage were placed on A.C.D. blood to be used for extracorporeal circulation.

Emergency use of extracorporeal circulation for pulmonary embolectomy and the temporary support of the heart after major myocardial infarctions is another way in which converted A.C.D. blood offers an advantage over fresh

fresh heparinised blood.

The theoretical disadvantages of the use of large volumes of converted A.C.D. blood for cardio-pulmonary bypass lie in the large initial acid load, the dilution of the blood by 120 ml. of fluid in each unit of 540 ml., and in the sodium load imposed by the 33.8 mEqu. of sodium also contained in each unit. The initial acidity of A.C.D. is of small importance for the reasons fully discussed in the section devoted to acid-base balance. Certainly where citrate metabolism is normal and high flow perfusion is maintained at all times, correction of the acidity of donor blood as suggested by Beer (Beer 1959) and Bucherl (Bucherl et al., 1959) is probably not necessary. If sodium bicarbonate was used to restore pH and standard bicarbonate of the priming volume to normal, the net effect would be to increase the sodium load - an undesirable event in patients with cardiac problems. The results of coronary perfusion with buffered A.C.D. blood, admittedly insufficiently recalcified to produce heparin equivalence, suggest that myocardial function could be improved by the addition of alkali if significant metabolic acidosis persisted, say, at the end of a period of extracorporeal circulation. It is suggested, therefore, that with accurate acid-base control of whole body perfusions with converted A.C.D. blood, the addition of sodium bicarbonate could be limited to correction of residual acidosis at the end of bypass. In infants and small children, the degree of metabolic acidosis possible during perfusion with both A.C.D. blood and day-old heparinised blood may be deleterious because of the limited buffering power of their extracellular fluid space. In these

these patients, truly fresh, well-buffered heparinised blood would be the perfusion medium of choice.

Haemodilution with A.C.D. solution has no serious significance to the patient undergoing extracorporeal circulation. Indeed since 1960 (Neptune et al., 1960; Zuhdi et al., 1960; Paton et al., 1964) haemodilution by up to 20 ml. per Kilogram of body weight has come to be an accepted practice in many centres. The extra fluid may be looked upon as representing the patients' fluid requirements for the day. It will seldom be greater than 1,500 ml. - the A.C.D. solution content of just under 7 litres of British banked blood - and will usually be considerably less.

The few minor changes seen in the plasma electrolytes during and after perfusion with converted A.C.D. blood are believed to be the result of ionic shifts according to the rules of the Donnan distribution law. They were never of a magnitude likely to be clinically significant i.e. they did not require correction by appropriate intravenous therapy.

The haematological findings are of interest because they clearly show the leucocytosis and thrombocytopenia now known to be non-specific responses to any major surgical trauma (Pepper and Lindsay, 1960).

Following upon the first case reported here, a further nine patients perfused with converted one-day-old A.C.D. blood were similarly investigated and compared with eight patients in whom heparinised blood was used. The changes seen in Case 1 were quite typical of the entire A.C.D. group and the overall picture was one of close similarity between the A.C.D. and

and heparin groups (Foote et al., 1960; ibid, 1961). Since that time, the use of converted A.C.D. blood up to five days old has become widespread, particularly in the United States of America where the difficulties of procurement of vast volumes of fresh heparinised blood are greater than in this country (Maloney : personal communication 1965).

The full implications of this study cannot yet be finally assessed. The data on recalcification of citrated blood and the coagulation studies have led to the clinical use of converted A.C.D. blood for extracorporeal circulation. It may be that the investigations of the cardiac effects of coronary perfusions with hyperkalaemic and cold blood, and of the effects of correction of acid-base disturbances on myocardial function, shed some light on fundamental aspects of cardiac physiology. If the findings here are accepted, they must have an important bearing on the proper management of massive and rapid intravenous blood transfusions. Clearly acid-base control of the shocked patient assumes major significance when one remembers that a fairly large increase in cardiac force can be generated by the correction of metabolic and respiratory acidosis. The fear of producing cardiac arrest or malfunction by using cold blood for transfusion (as long as it is adequately covered by the simultaneous administration of calcium; and total body hypothermia is not allowed to occur (Boyan et al., 1964)) need not trouble the clinician. In the greatest proportion of coronary perfusions with cold blood an actual increase in cardiac contractility was measured. One must look elsewhere than the heart, therefore, for

for an explanation of the dangers of cold blood transfusions. They would seem to be inseparable from the hazards of total body hypo-:thermia below 28°C. Similarly also, the place of hyperkalaemia in bringing about cardiac arrest during ultra-rapid, old-blood transfusions is brought into perspective by the lack of cardiac response to perfusion with blood containing up to 14.45 mEqu. of potassium per litre.

S U M M A R Y

The relative importance of hypocalcaemia, acid-base disturbances, hyperkalaemia, and cold, in producing cardiac arrhythmias or diminished contractile force of the myocardium was assessed by a direct coronary perfusion technique in dogs.

Heparinised or A.C.D. (acid-citrate-dextrose) blood with various electrolyte additions was used in 128 perfusions. When 0.686 ± 0.13 ml. of calcium chloride 20% solution B.P. were added to 50 ml. A.C.D. blood, equivalent effects to those of heparinised blood were produced on ventricular contractile force (V.C.F) measured by a Walton-Brodie strain gauge arch sutured to the ventricle, and the electrocardiogram. Correction of the metabolic acidosis or the respiratory acidosis normally present in A.C.D. (or both simultaneously) resulted in a moderate (14-31 per cent) improvement in V.C.F. Perfusion with recalcified A.C.D. or heparinised blood cooled to 10°C, resulted in an increase in V.C.F. (average 34 per cent with A.C.D. and 40 per cent with heparinised blood). Hyperkalaemia up to 14.45 mEq./L had no statistically significant effect on V.C.F. or E.C.G.

Calcium chloride solution was found to have antiheparin effects *in vitro*, but these were insignificant when heparin concentrations of 5,000 units per litre were used. No reversal of heparinisation by intravenous calcium was found *in vivo*.

A.C.D. blood "converted" by the addition of 5,000 units of heparin per litre and 12 ml. of 20 per cent calcium chloride solution B.P. per litre, was found to be a satisfactory medium for priming heart-lung machines before extracorporeal circulation in dogs and man.

Details were given of the first fully documented human whole body normothermic perfusion in which only converted A.C.D. blood was used. The intrinsic metabolic acidosis of the donor blood had largely disappeared one hour after the mixing of recipient and priming bloods. Otherwise no important changes in biochemical (plasma potassium, sodium, haemoglobin, and chloride) haematological (white blood counts, platelet counts, haematocrits, and clotting times) or haemodynamic (blood pressure, venous pressure, and electro-cardiogram) parameters were seen, other than those which result from any major surgical procedure.

The advantages and disadvantages of this system for priming extracorporeal circuits and the implications of the findings in the coronary perfusion experiments, in relation to massive and rapid blood transfusions, were discussed.

APPENDIX

FIGURES 1 - 26

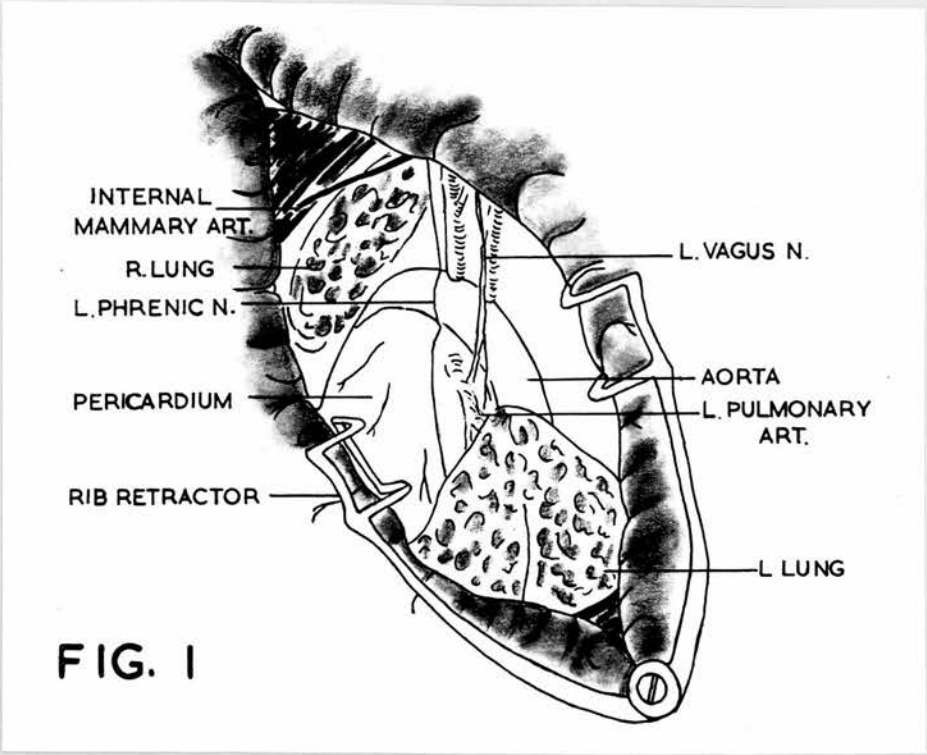

FIG. 1

Drawing of operative exposure for coronary profusion technique. Thoracotomy through the fourth left intercostal space.

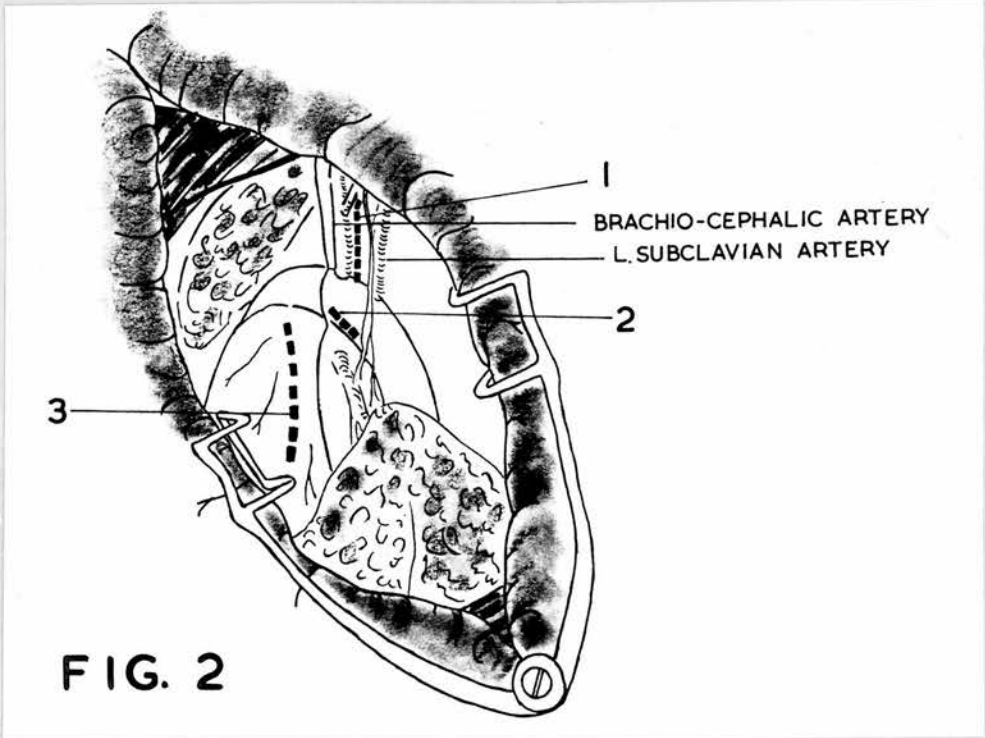

FIG. 2

Pleural incisions for exposure of (1) brachiocephalic artery, (2) aortic arch, (3) left atrial appendage.

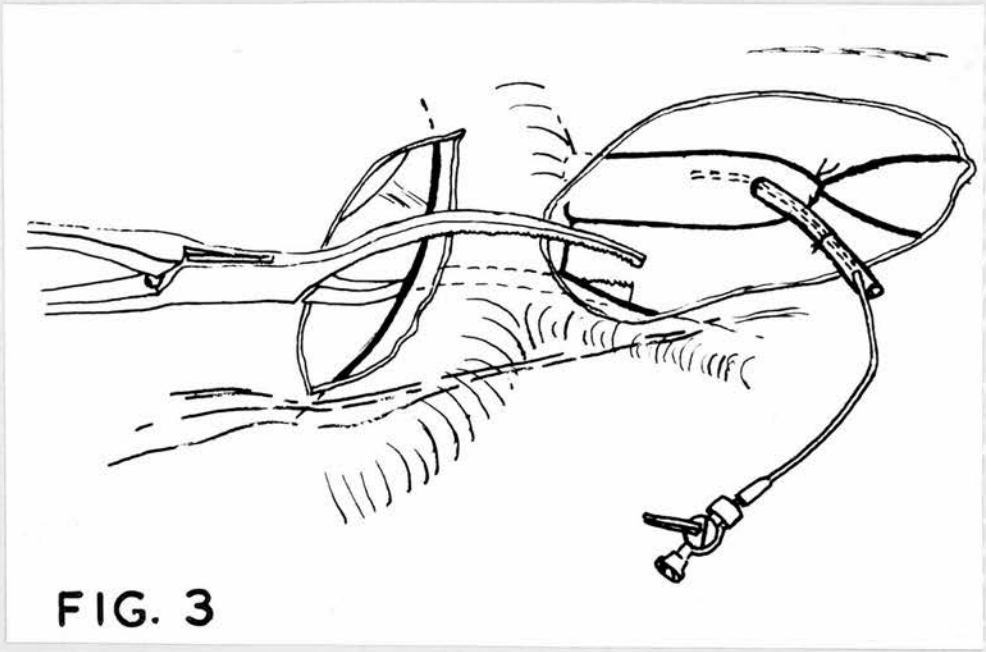

FIG. 3

Drawing showing position of aortic clamp, and cannula in brachiocephalic artery.

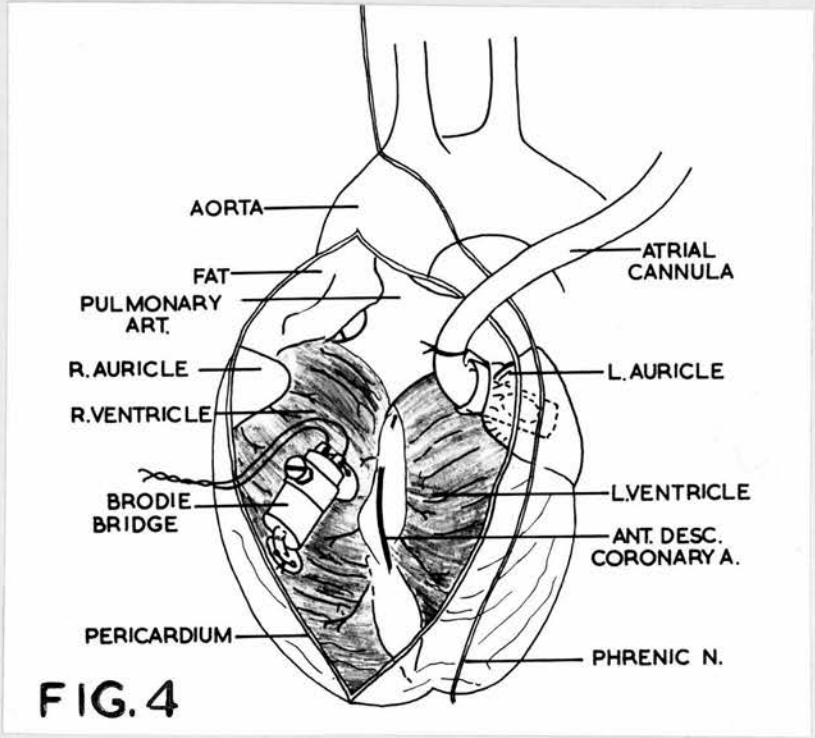

FIG. 4

Strain gauge arch sutured to right ventricle avoiding incorporation of coronary vessels in stitches. Cannula within left atrium.

FIG. 5

Brodie bridge, extension wire and Wheatstone bridge as used with the Devices 8-channel recorder in the coronary perfusion experiments described in the text.

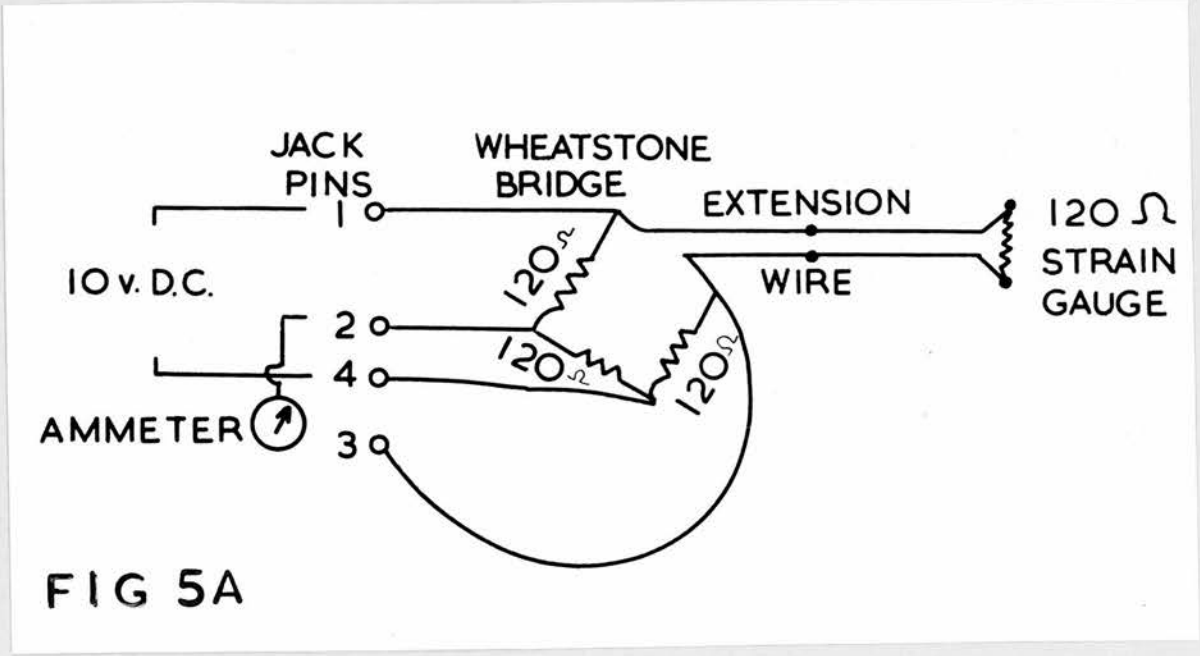

FIG 5A

Electrical circuit of device in Fig. 5. The pin numbers (1-4) refer to the plug-in jack for the Devices 8-channel recorder. The latter supplies the 10 volt current and ammeter (pen recorder).

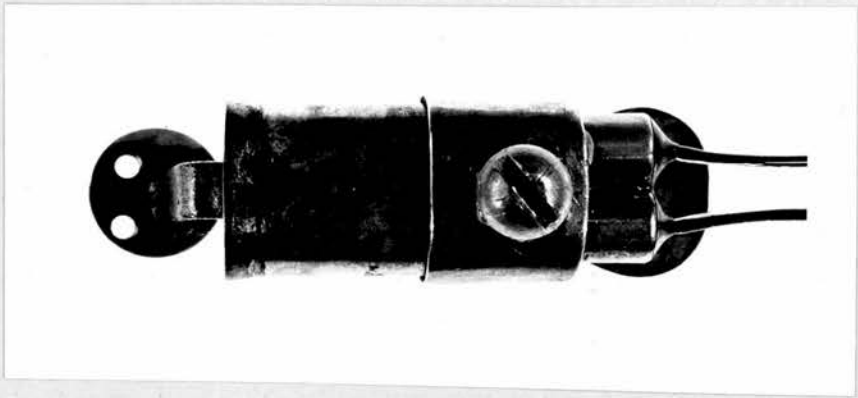

FIG. 6

Walton-Brodie 120 ohm strain gauge arch (2 views) as supplied by Mr. John A. Warren, Post Office Box 412, Charleston, South Carolina, U.S.A.; approximately twice normal size.

Refs. Boniface et al. 1956; Cotton, 1953; Cotton & Bay, 1956; Cotton and Maling, 1957.

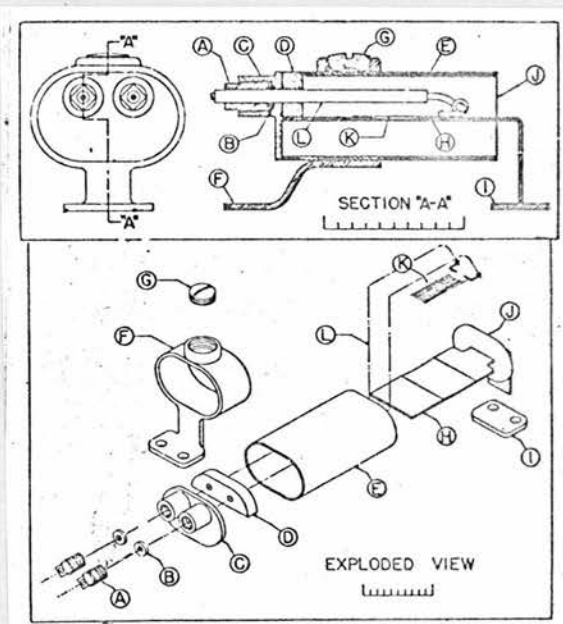

FIG. 6. Strain gauge arch with coil enclosed in brass tubing encasement. Above—photograph about twice actual size. Below—diagrammatic, sectional and exploded views with one cm scale. Parts designations as follows: (A) Stuffing box gland. (B) Stuffing box packing. (C) Body end integral with stuffing boxes. (D) Stop preventing upward movement of (H). (E) Tubular body or encasement. (F) Movable foot. (G) Set screw. (H) Elastic metal strip integral with rigid leg. (I) Foot. (J) Flexible metal diaphragm. (K) Strain gauge coil. (L) Lead wires.

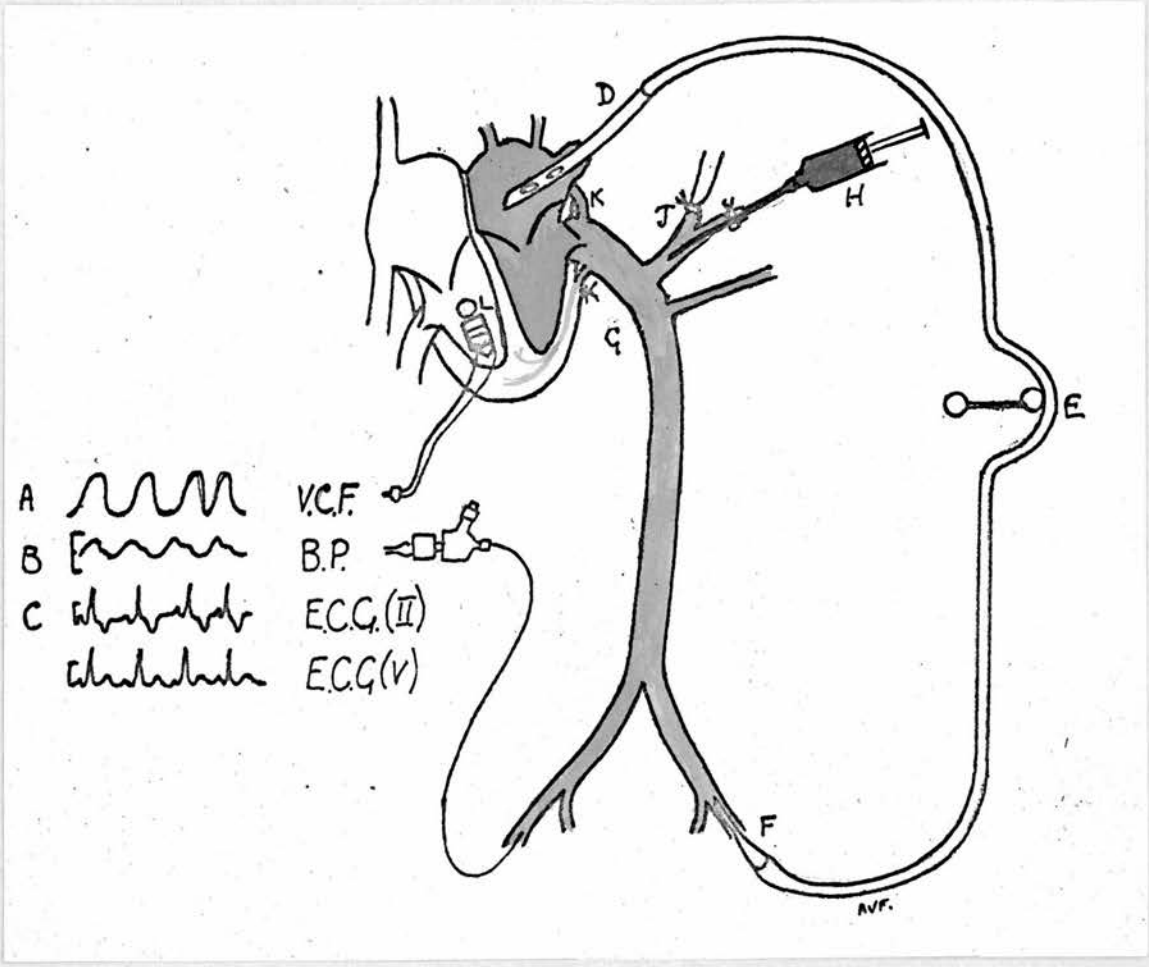

FIG. 7

Diagram of Circuit used for left-heart bypass and coronary artery perfusion.

A - Ventricular Contractile Force
B - Systemic Blood Pressure
C - E.C.G. (Lead II)
D - Left Atrial Cannula
E - Pump
F - Arterial Cannula
G - Aortic Clamp
H - Blood for Coronary Perfusion
J - Brachiocephalic Artery
K - Coronary Artery
L - Walton-Brodie Strain Gauge

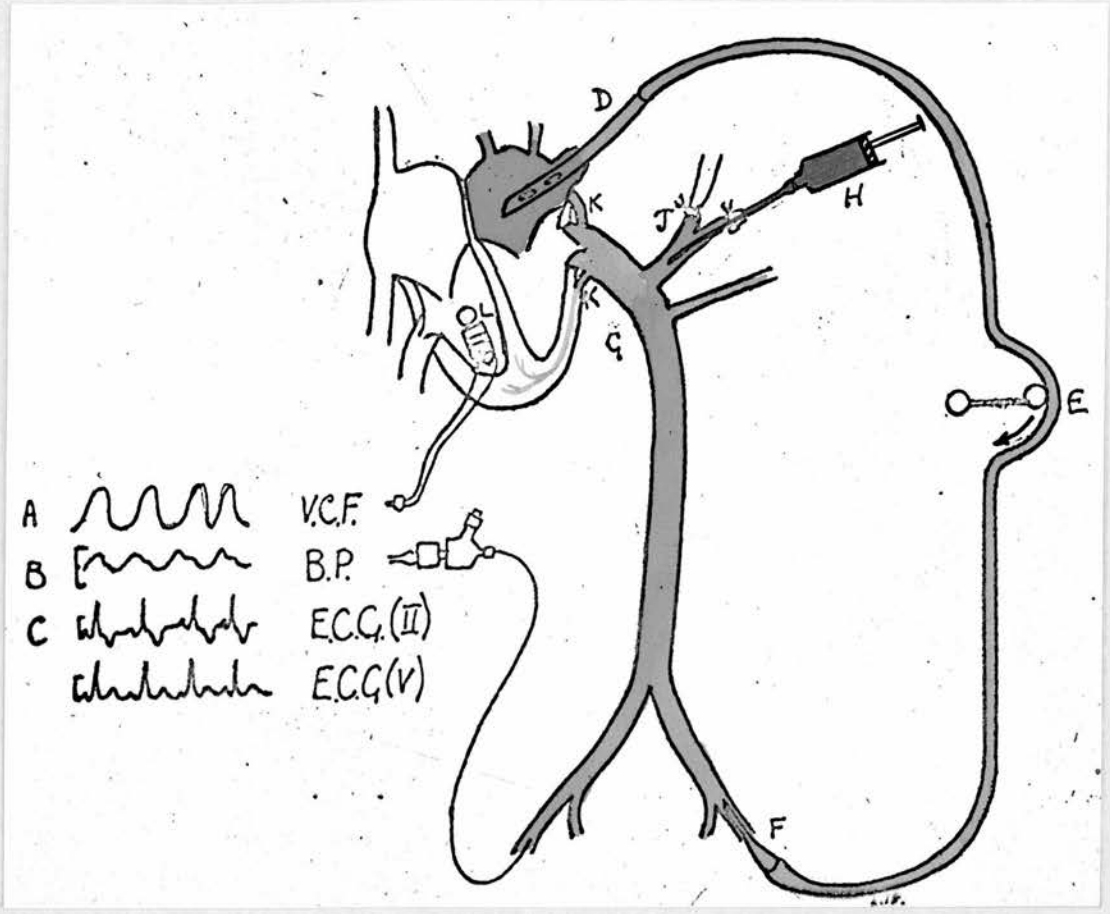

FIG. 8

Pump turned on and adjusted to bypass entire left atrial venous return to femoral artery.

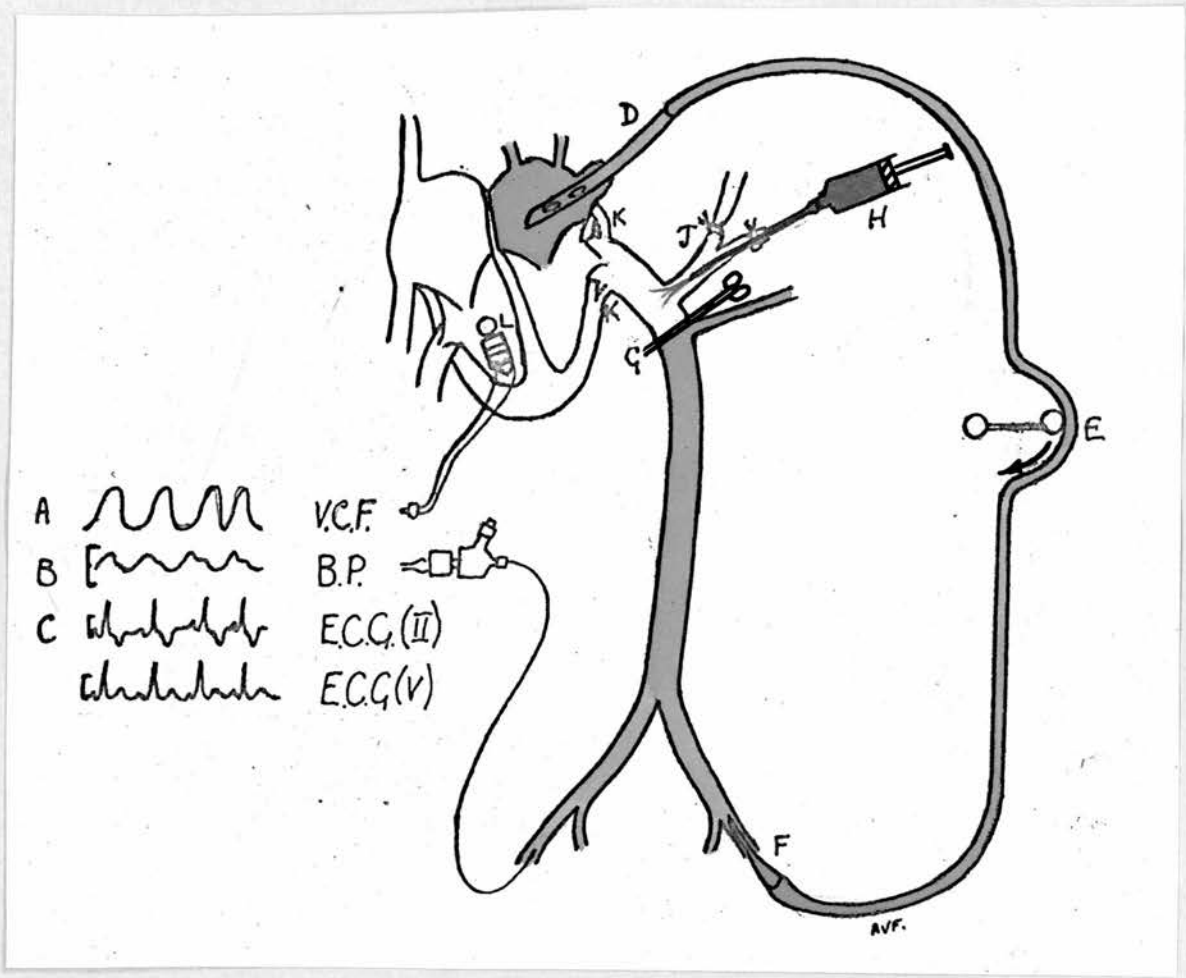

FIG. 9

Aorta cross-clamped : proximal aorta empty : perfusion ready to begin.

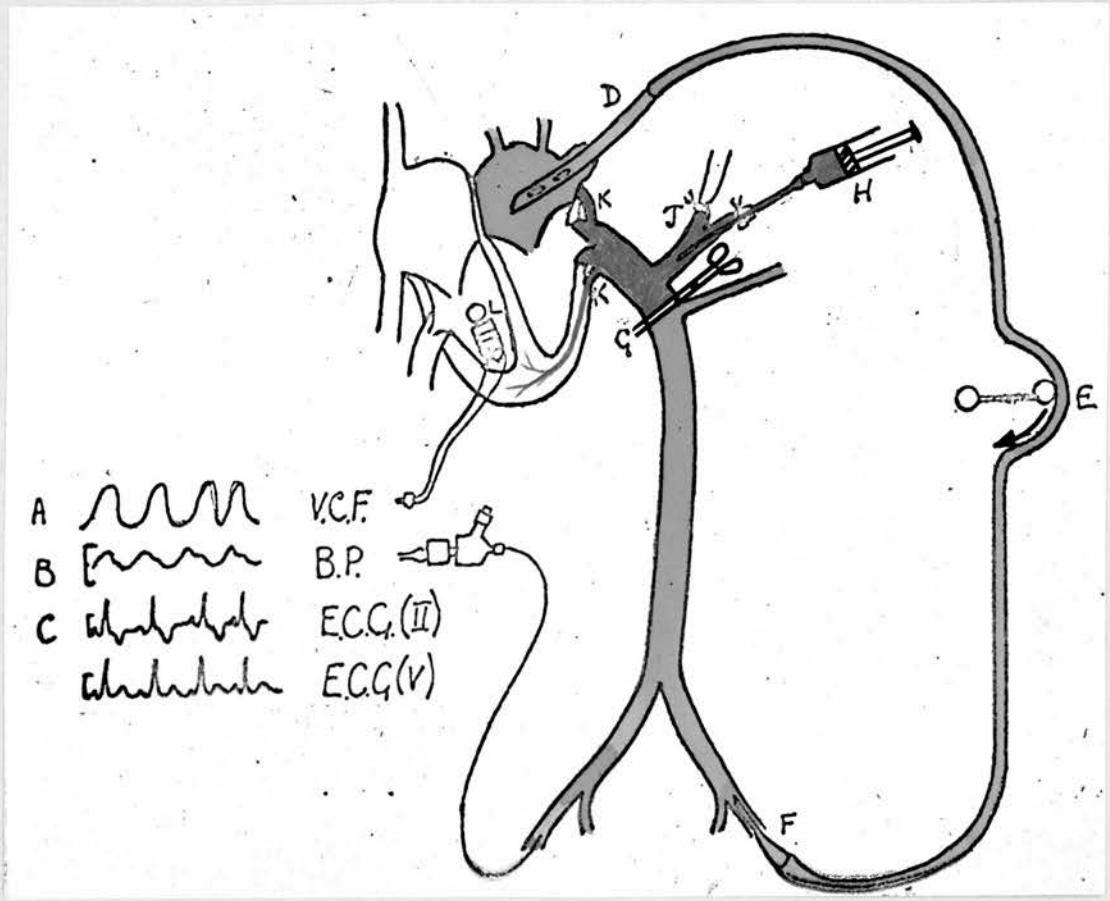

FIG. 10

Coronary perfusion in progress.

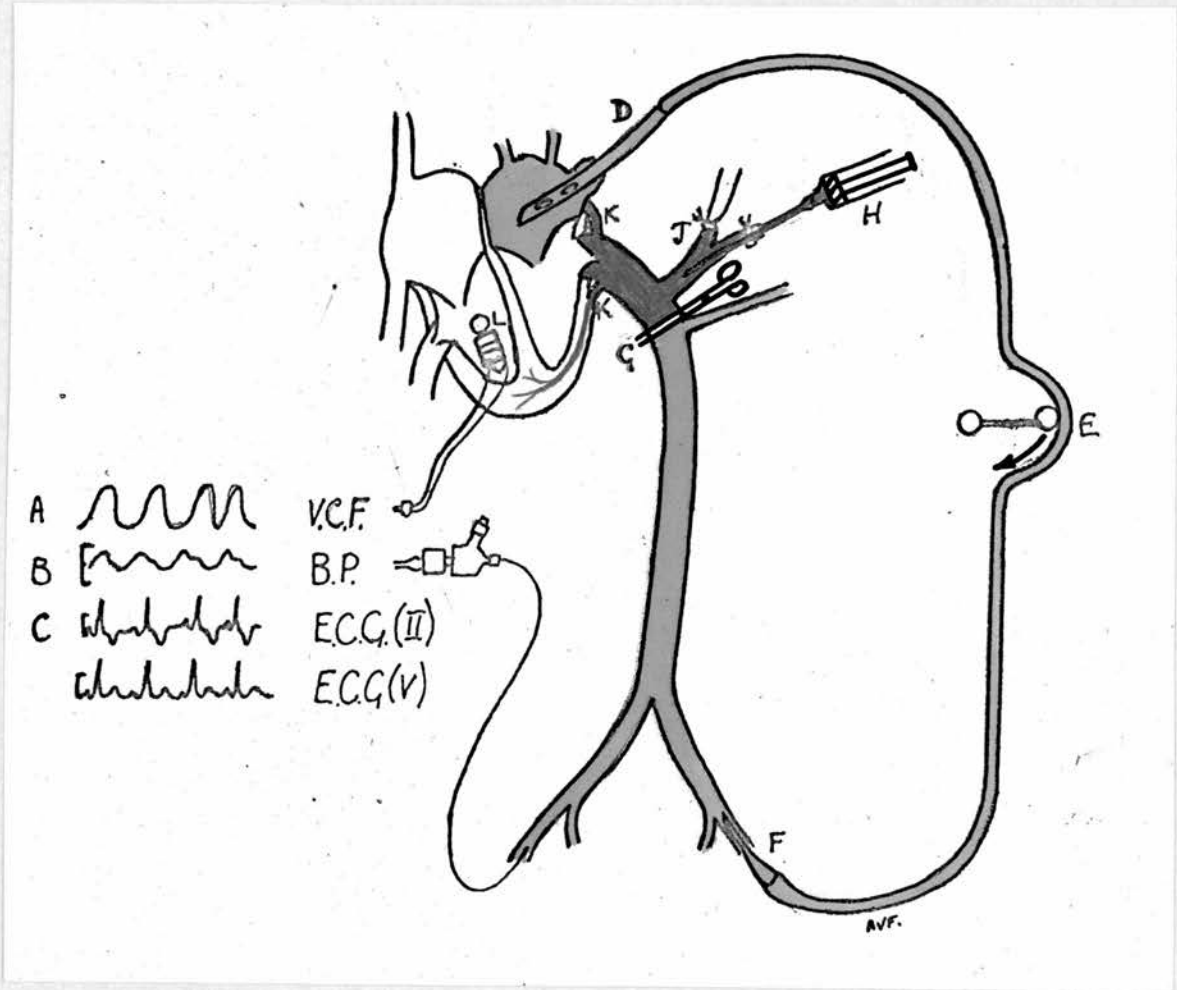

FIG. 11

Coronary perfusion complete after 60 seconds.

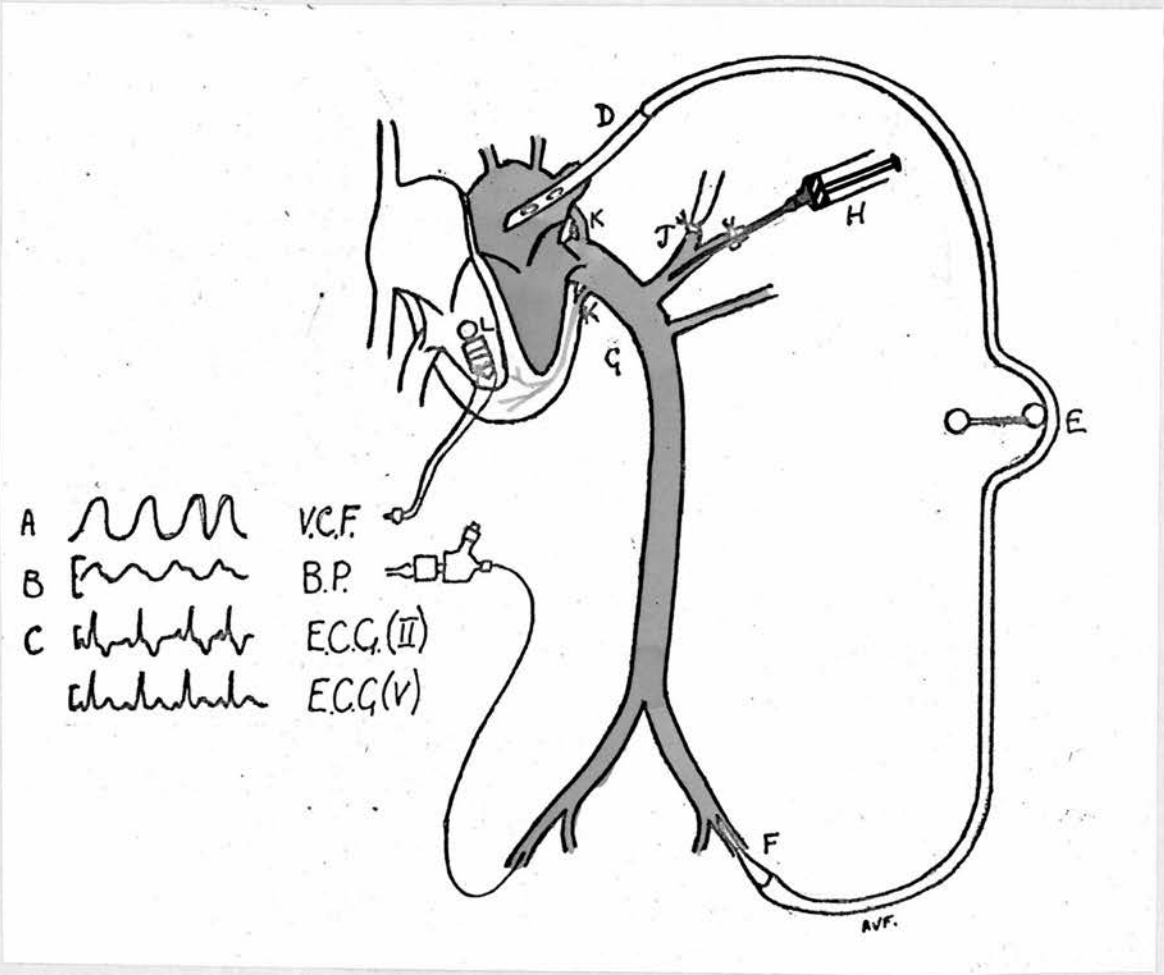

FIG. 12

Pump stopped : aortic clamp removed. Preparation allowed 15 to 20 minutes for recovery before the cycle (Figs. 7-12) was repeated.

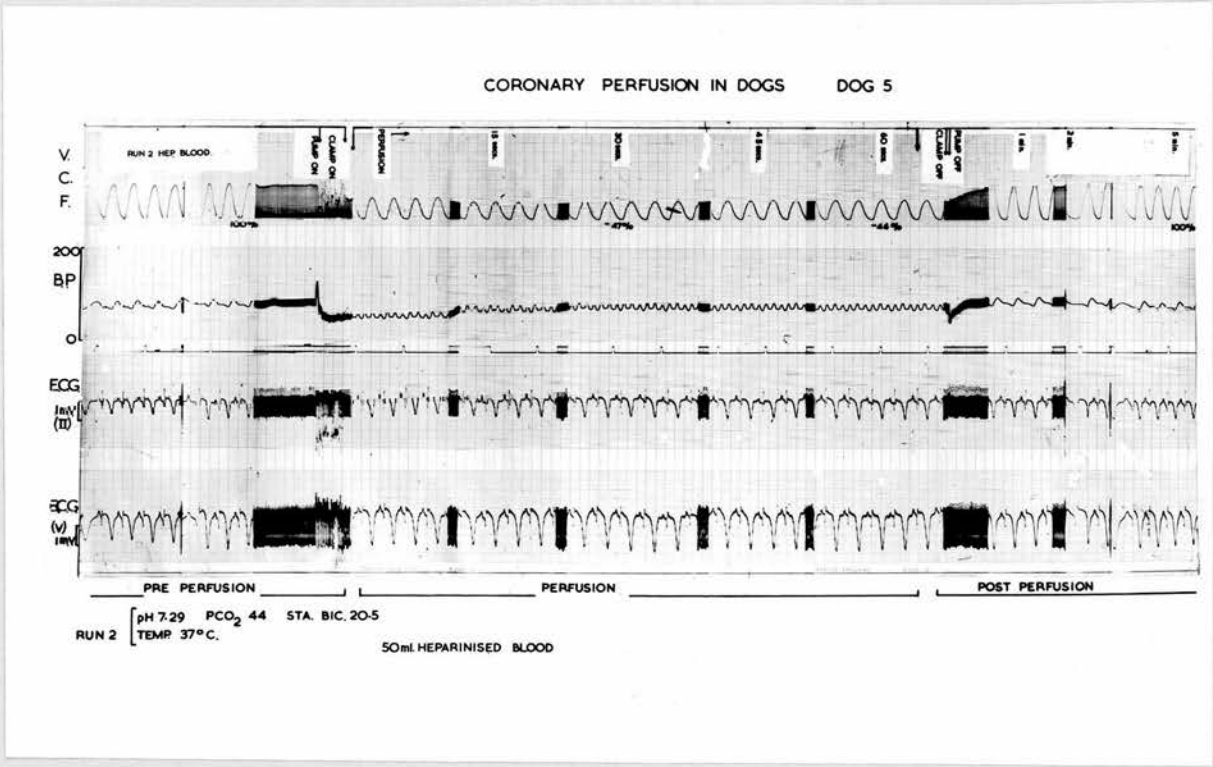

FIG. 13

Actual tracing of perfusion 5(2) showing the four simultaneous records (V.C.F., aortic blood pressure, E.C.G. lead II, E.C.G. lead V_5). Note: the oscillations in blood pressure during perfusions are due to the pump and are asynchronous with the heart beat.

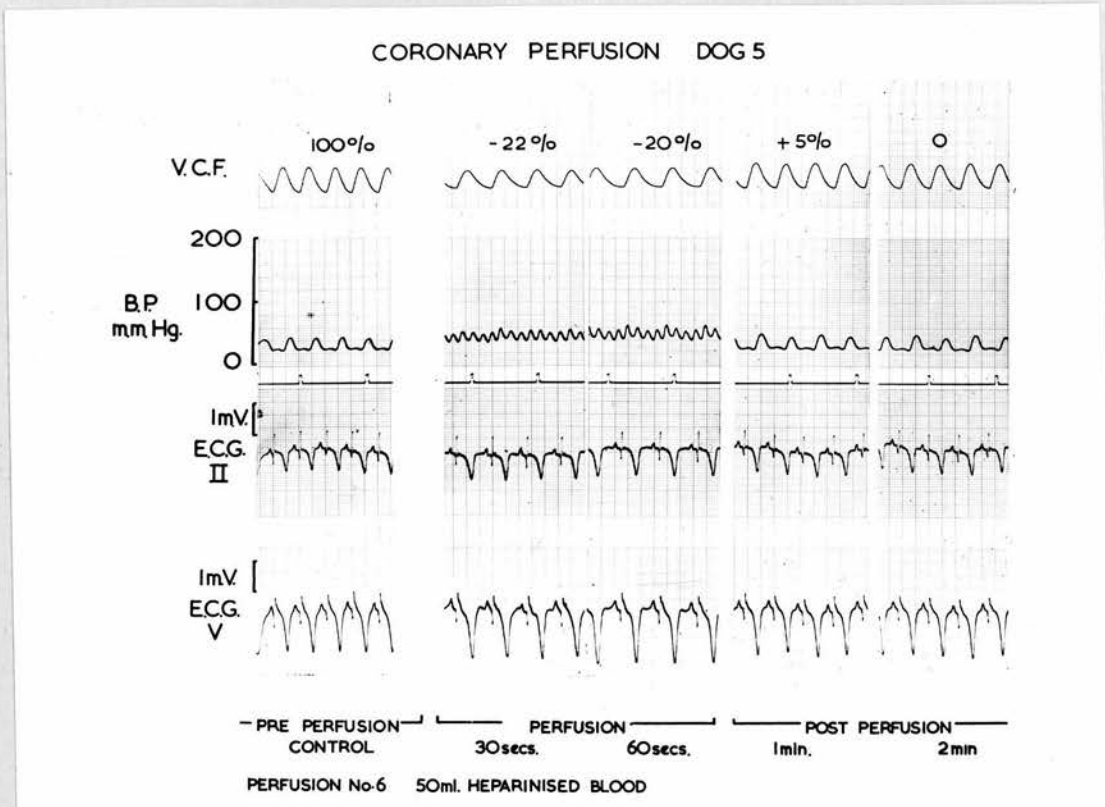

FIG. 14

Abstracts from record of perfusion (No. 5(6)), using heparinised blood. The control period, perfusion and recovery period are shown. The percentage changes in V.C.F. from the control period (= 100%) are given after 30 seconds and 60 seconds of perfusion. No alterations in E.C.G. occurred.

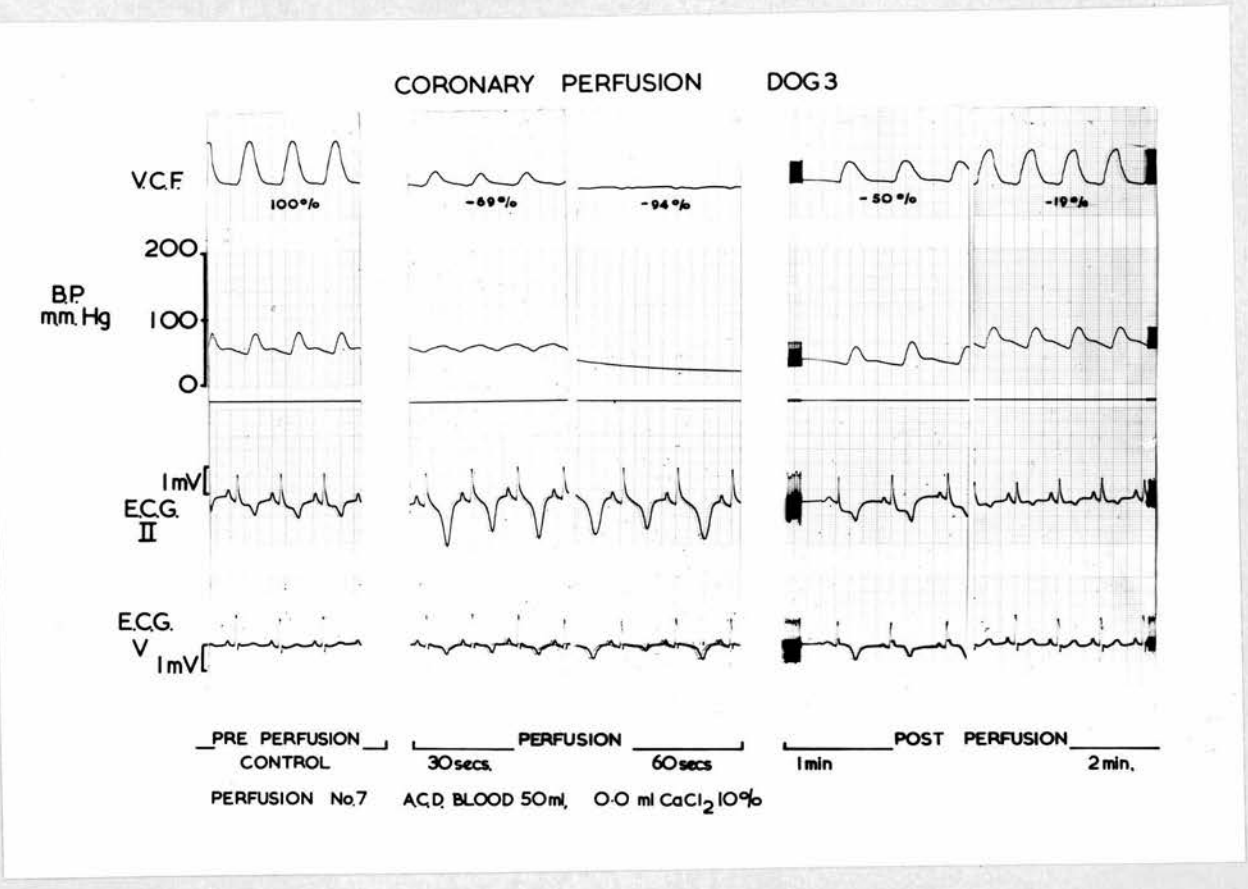

FIG. 15

Abstracts from record of perfusion 3(7) using A.C.D. blood with no calcium added. Note the continuation of high voltage E.C.G. tracings while V.C.F. is depressed by 94% after 60 seconds of perfusion. T-wave depression and Q-T interval elongation can be measured.

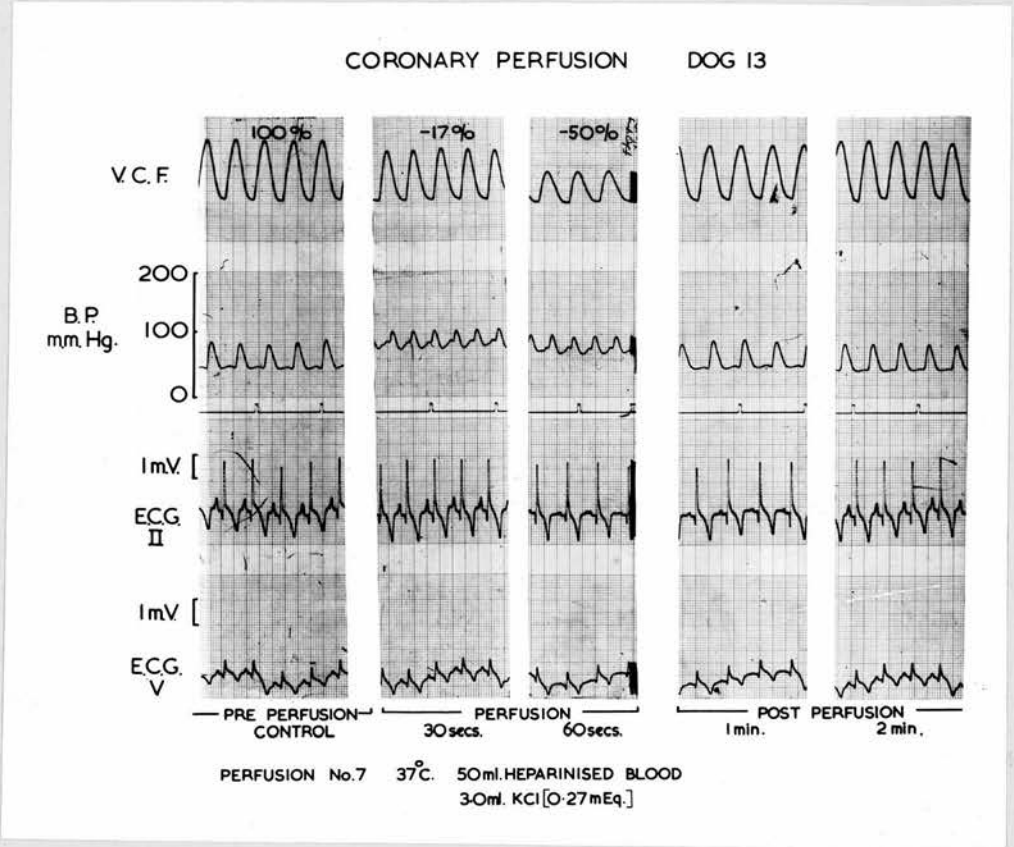

FIG. 16

Perfusion 13(7): Heparinised blood containing added potassium. Measured plasma potassium of perfusate was 11.5 mEqu./L. A decrease in V.C.F. of 50 per cent followed 60 seconds of hyperkalaemic perfusion.

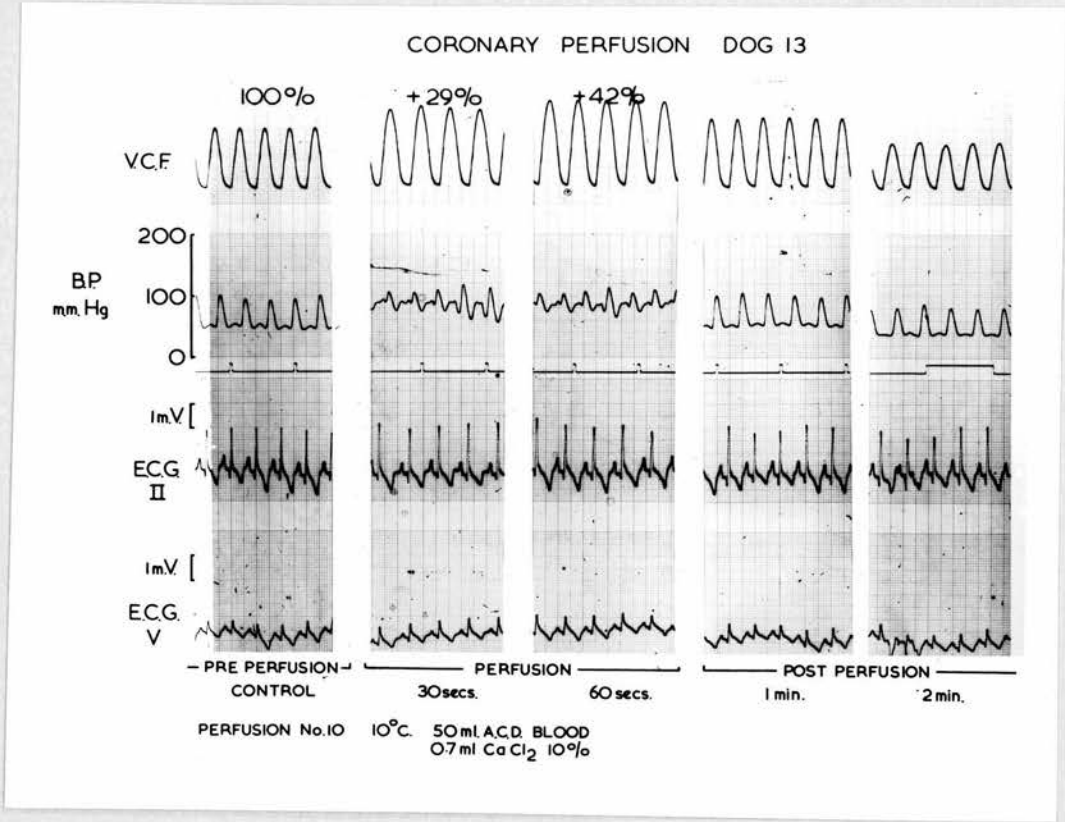

FIG. 17

Perfusion 13(10) A.C.D. blood at 10°C containing 0.7 ml. calcium chloride solution in 50 ml. blood.
An increase in V.C.F. of 42 per cent followed 60 seconds of hypothermic perfusion.

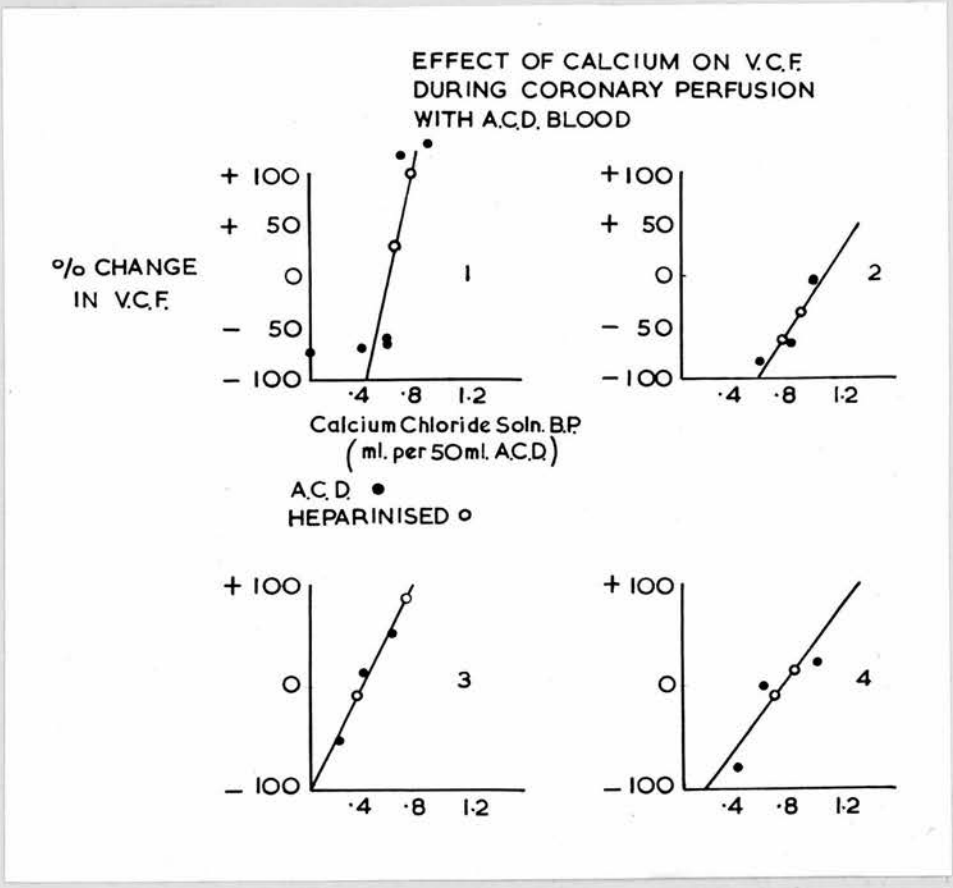

FIG. 18

Heparin equivalence Points. (Dogs 1-4)
Changes in V.C.F. from control are plotted against additions of 20% Calcium Chloride solution (ml. per 50 ml.) to A.C.D. blood before perfusions. See text.

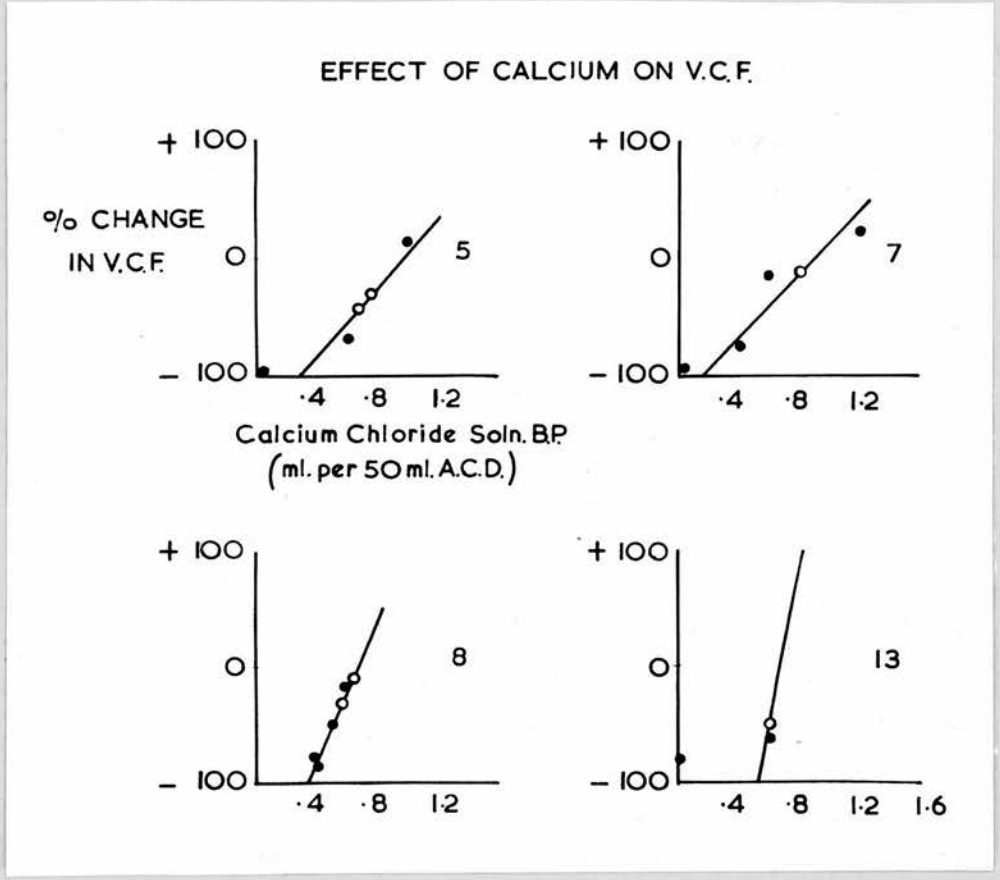

FIG. 19

Heparin Equivlance Points (Dogs 5, 7, 8, 13).
Note: the regression line in dog 13 merely joins the two points representing the addition of 0.6 and 1.6 ml. of Calcium Chloride, and may not be fully acceptable.

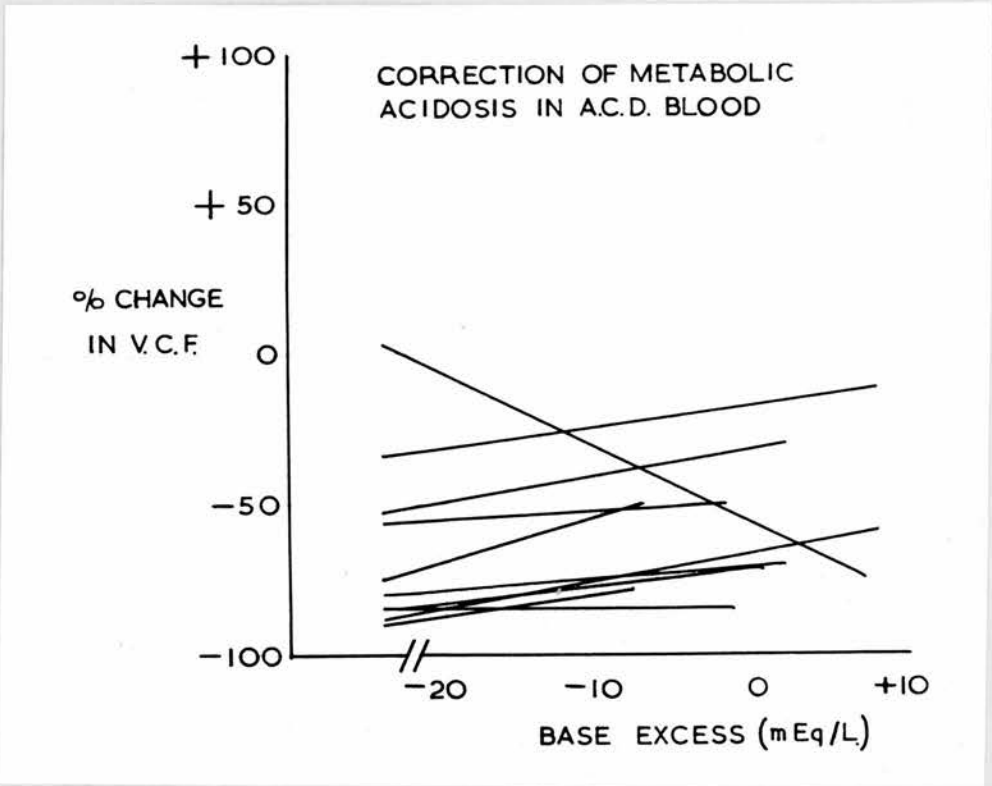

FIG. 20

The Effect of V.C.F. of Correction of Metabolic Acidosis in A.C.D. Blood before Coronary Perfusion. Each line connects the changes in V.C.F. after 60 seconds of perfusion in pairs of perfusions matched for type of blood, calcium addition, plasma potassium concentration and PCO_2.
Note: hypernatraemia resulted from correction of metabolic acidosis with 8.4% sodium bicarbonate solution.

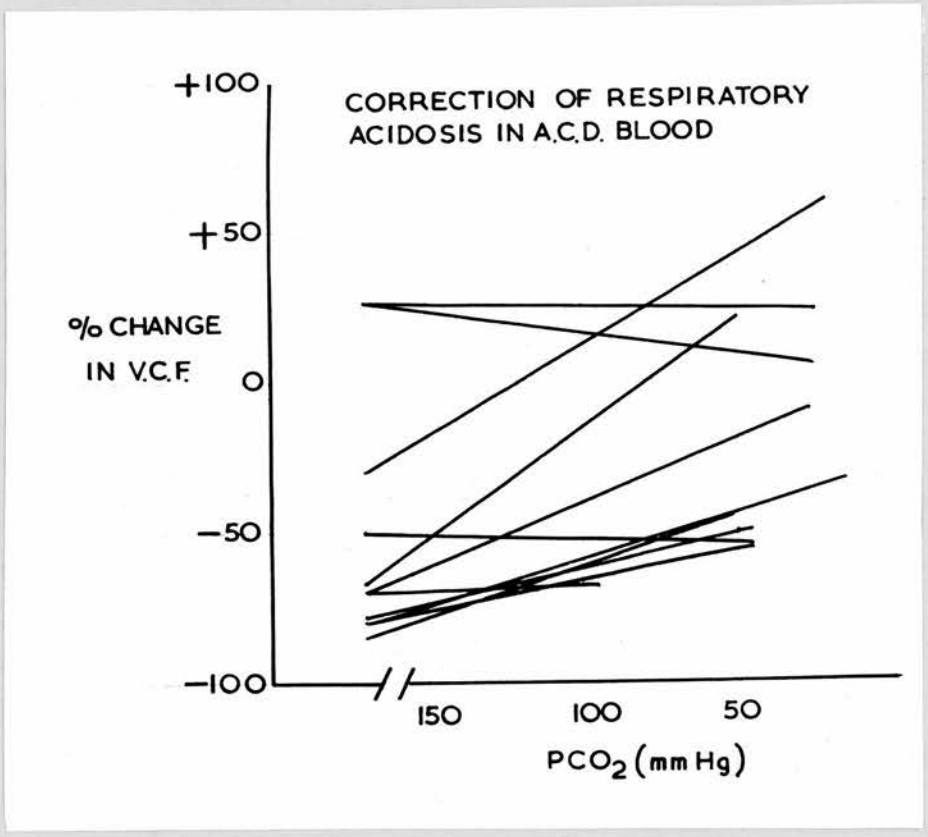

FIG. 21

The Effect of V.C.F. of Correction of Respiratory Acidosis in A.C.D. Blood before Coronary Perfusion.
Each line connects the changes in V.C.F. after 60 seconds of perfusion in pairs of perfusions matched for type of blood, calcium addition, plasma sodium and potassium concentrations, and base excess.

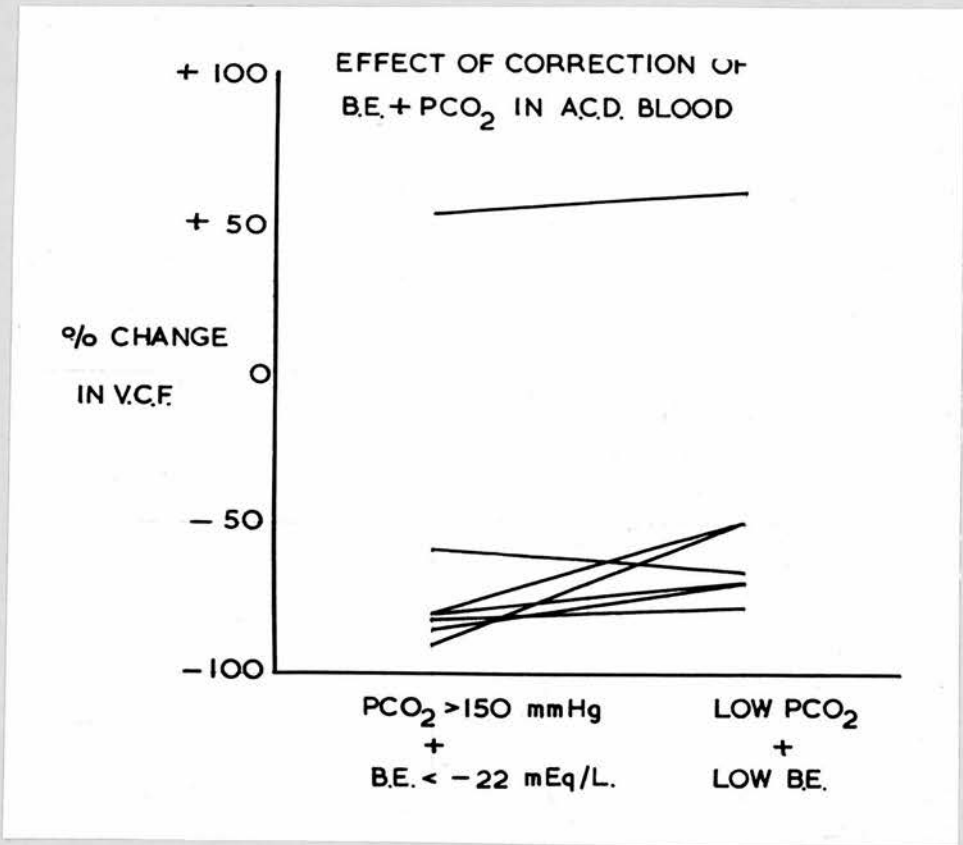

FIG. 22

The Effect of V.C.F. of Correction of both Respiratory Acidosis and Metabolic Acodisos of A.C.D. Blood before Cpronary Perfusion.

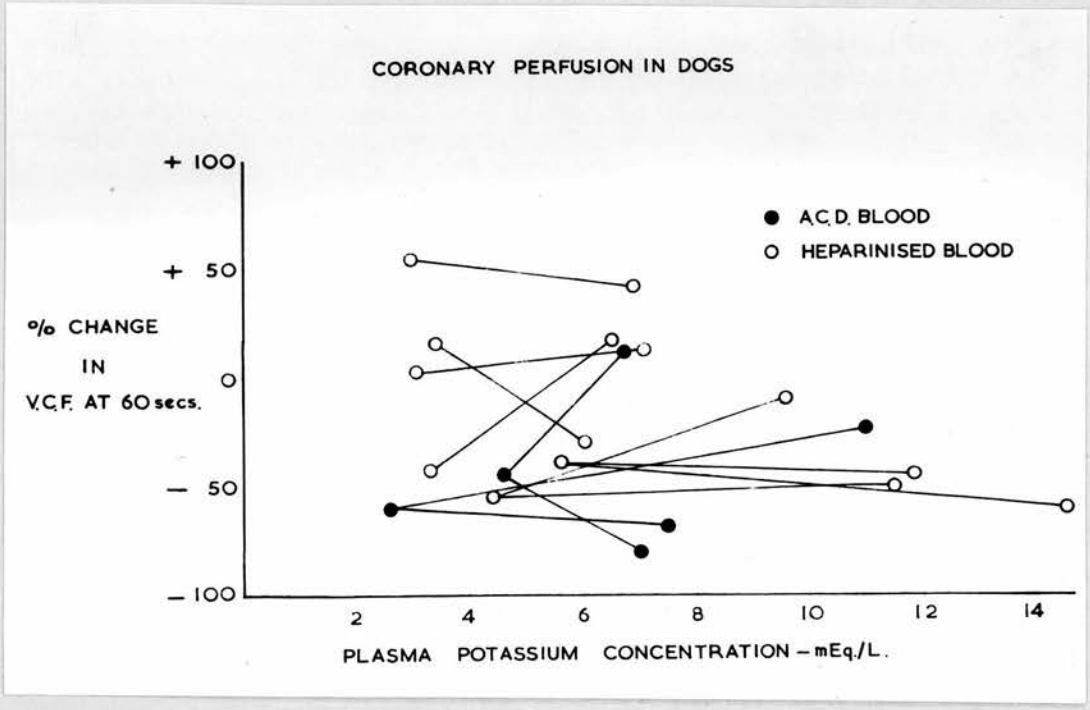

FIG. 23

The Effect of V.C.F. of Hyperkalaemic Blood Perfusing the Coronary Arteries for 60 seconds.
Each line connects the percentage changes in V.C.F. in pairs of perfusions matched for type of blood, calcium addition, base excess, PCO_2 and plasma sodium concentration.

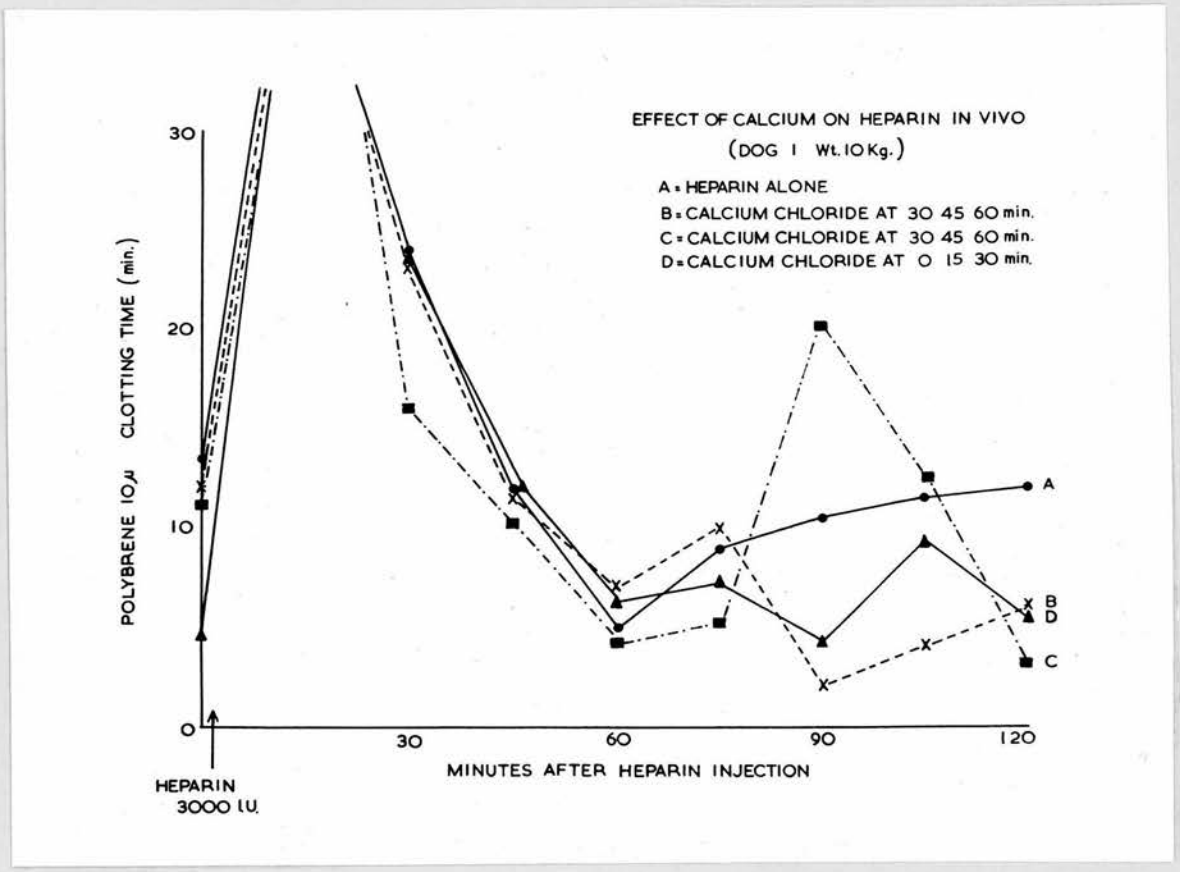

FIG. 24

The Effect of Calcium on Polybrene (10μ) Clotting Time in a Heparinised Dog.
The same dose of heparin was used in all four experiments on the same dog. Calcium Chloride solution (20% B.P.) was given intravenously, at the times indicated in the figure, in three experiments.

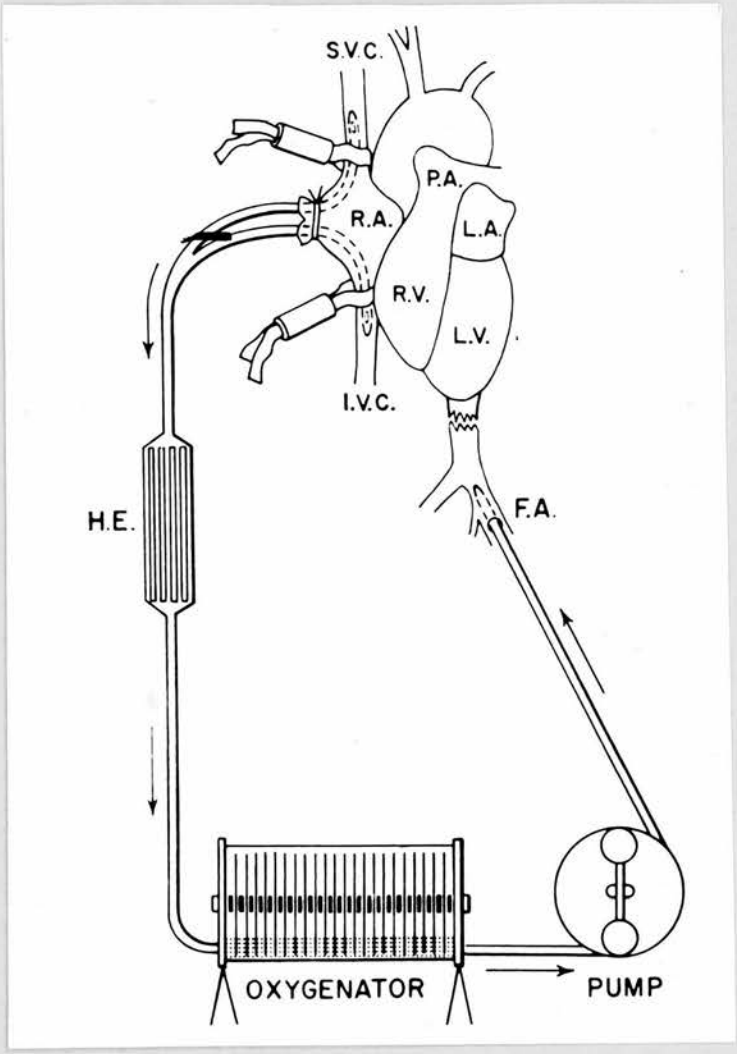

FIG. 25

Diagram of Extracorporeal Circuit used for Total Cardio-Pulmonary Bypass in Dogs.

S.V.C.	- Superior vena cava	;	R.A. - Right atrium;
P.A.	- Pulmonary artery	;	L.A. - Left atrium;
R.V.	- Right ventricle	;	L.V. - Left ventricle;
I.V.C.	- Inferior vena cava	;	F.A. - Femoral artery;
H.E.	- Heat exchanges	;	
Oxygenator	- Kay-Cross 16 inch rotating disc oxygenator		
Pump	- De Bakey roller pump		

The heat exchanger, though not strictly necessary, maintained normothermia. The circuit was identical with that used in human extracorporeal circulation except that, in the latter, a heating coil around the oxygenator replaced the heat exchanger.

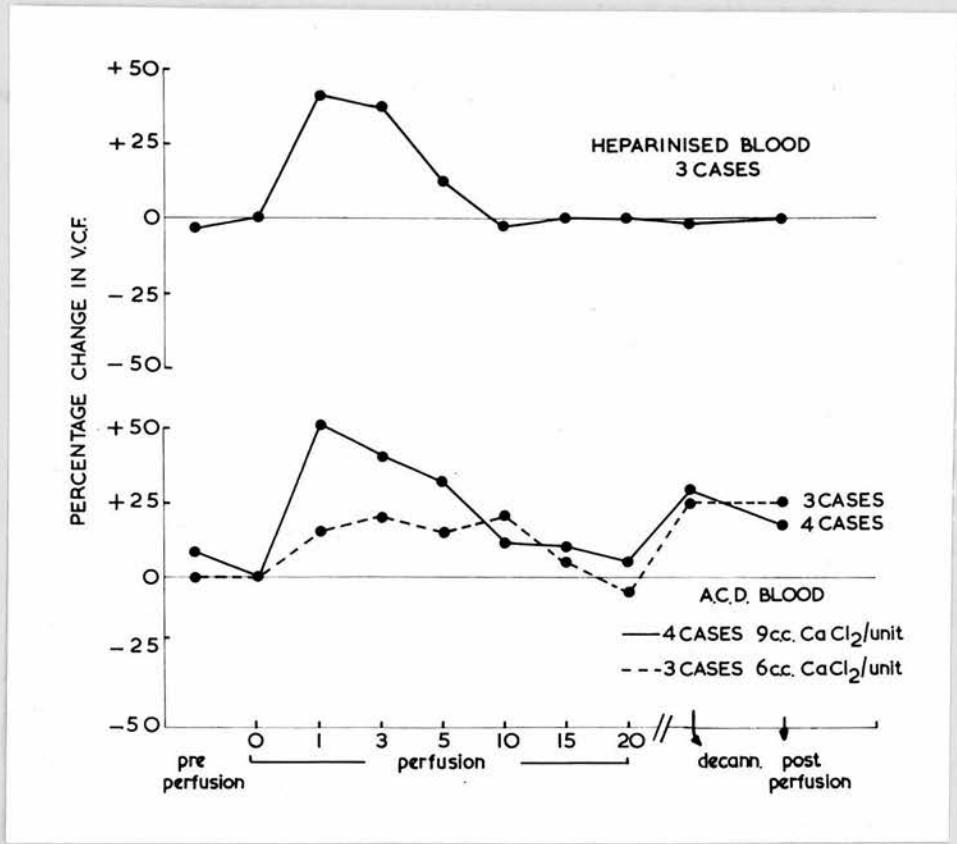

FIG. 26

Changes in Ventricular Contractile Force during Total Cardio-Pulmonary Bypass in Dogs.
3 groups of dogs are compared before, during and after bypass, using:

(1) **Heparinised blood**
(2) **Heparinised A.C.D. blood containing 6 ml. 10% calcium chloride solution U.S.P. per 500 ml.**
(3) **Heparinised A.C.D. blood containing 9 ml. 10% calcium chloride solution U.S.P. per 500 ml.**

TABLES 1-43

DOG	Percentage Change in V.C.F. By Perfusion with 50 ml. Blood after 60 secs.												Heparin Equivalence Point (ml. Ca Cl2)
	Hep. I	Hep. II	A.C.D. Blood + Calcium Chloride Soln. (20% B.P.) ml. as Below										
			0	0.2	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.6	
1	+30	+100	- 72		-71		-63 -61	+120		+130				.64 .74
2	-33	- 67					-85		-67		- 4			.75 .88
3	-10	+ 88	- 94	-50	+14		+53							.72 .35
4	+18	- 9	- 93		-80 -82		0					+22		.68 .82
5	-40	- 30	-100				-70				+14			.67 .75
7	-10	-	- 95		-90 -76		-15					+22		.80
8	-31	- 11			-85 -80	-50	-17							.56 .64
13	-51	-	- 80				-60						+310	.60
Mean														0.686
Range														0.35-0.88
S.D.														± 0.13

TABLE I

Effect of Calcium on Percentile Changes in V.C.F. produced by Coronary Perfusion with A.C.D. Blood.
For Heparin Equivalence Points see Figs. 19 and 18

TABLE 2

CORRECTION OF ACIDOSIS IN A.C.D. BLOOD

Dog.	Cont. No.	Test No.	Cont. pH	Test pH	Cont. PCO_2	Test PCO_2	Cont. B.E.	Test B.E.	Cont. V.C.F.	Test V.C.F.	△ V.C.F.	Cont. T II.	Test T II	Cont. Q-T	Test Q-T	Cont. Rate	Test Rate
A																	
2	(3)	(2)	<6.6	6.73	>150	>150	<-22	-1.5	-85	-85	0	-1	--	+1	+2	-40/ 120	-41/ 115
3	(4)	(3)	<6.6	6.81	>150	>150	<-22	+2.1	+53	-30	+23	-	-5	+1.5	+2.5	- 4/ 98	-10/ 100
7	(3)	(5)	<6.6	6.6	>150	>150	<-22	-8	-90	-78	+12	+7.5	+8.5	+1	+2	-53/ 130	-46/ 126
8	(4)	(6)	6.3	6.88	>150	>150	<-22	+2	-85	-70	+15	+ .5	- .5	+2	+3	-26/ 87	-24/ 80
8	(7)	(5)	6.6	7.59	16	27	<-22	+8	-33	-11	+22	--	- .5	+ .5	+ .5	+ 9/ 71	- 0/ 66
8	(9)	(6)	6.3	6.88	>150	>150	<-22	+2	-80	-70	+10	-1	- .5	+2	+3	+24/ 48	-24/ 80
12	(2)	(5)	6.25	7.00	>150	>150	<-22	+8	-88	-59	+19	+4.5	--	--	+2	+46/ 140	0/ 130
11	(2)	(4)	<6.6	7.05	>150	>150	<-22	+7	+ 3	-78	-75	+5.5A 1 - .5t.	0	0	+1.5	- 5/ 125	-12/ 130
4	(6)	(4)	6.76	7.70	45	15.5	<-22	-2	-56	-50	+ 6	--	-1	ES	+2	-31/ 125	-30/ 135
7	(6)	(4)	6.6	7.21	40	46	-22	-7.5	-75	-50	+28	--	-.5	--	+1	-30/ 130	-25/ 130
B																	
3	(3)	(2)	6.81	7.52	>150	21	+2.1	-3	-30	+60	+90	-5	+1	+2.5	+ .5	-10/ 100	- 0/ 105
4	(3)	(6)	6.32	6.76	>150	45	<-22	-22	-80	+56	+36	--	--	--	--	-30/ 135	-31/ 125
5	(3)	(4)	6.3	<6.6	>150	92	<-22	<-22	-70	-69	+1	-1	-2	+2	+2	-34/ 150	-30/ 150
6	(2)	(4)	6.35	6.5	>150	46	<-22	<-22	-50	-55	-5	-2.5	+ .5	+3	+ .5	-22/ 96	-16/ 96

TABLE 2 (Cont'd.)

CORRECTION OF ACIDOSIS IN A.C.D. BLOOD

Dog.	Cont. No.	Test No.	Cont. pH	Test pH	Cont. PCO_2	Test PCO_2	Cont. B. E.	Test B.E.	Cont. V.C.F.	Test V.C.F.	Δ V.C.F.	Cont. T II	Test T II	Cont. Q-T	Test Q -T	Cont. Rate	Test Rate
B.																	
7	(5)	(4)	6.6	7.21	>150	46	-8	-7.5	-78	-50	+ 28	+8.5	- .5	+2	+1	-46/ 126	-25/ 130
8	(6)	(5)	6.88	7.59	>150	27	+2	+8	-70	-11	+ 59	- .5	- .5	+3	+ .5	-24/ 80	0/ 66
8	(4)	(7)	6.3	6.6	>150	16	<-22	<-22	-85	-33	+ 52	+ .5	--	+2	+ .5	-26/ 87	+ 9/ 71
8	(9)	(7)	6.3	6.6	>150	16	<-22	<-22	-80	-33	+ 47	-1	--	+2	+ .5	+24/ 48	+ 9/ 71
11	(2)	(5)	<6.6	6.78	>150	25	<-22	<-22	+ 3	+26	+ 23	+5.5 A 1 -0.5t.	--	0	- .5	+ 5/ 130	- 5/ 125
11	(6)	(5)	<6.6	6.78	>150	25	<-22	<-22	+31	+26	- 5	+4 A 1 -1 t.	--	+1	- .5	+20/ 110	- 5/ 125
12	(2)	(4)	7.0	7.40	>150	50	+ 8	+ 6	-88	-67	+21	+4.5	- .5	+2	+2	-46/ 140	-35/ 135
C.																	
4	(9)	(5)	6.3	6.91	>150	114	<-22	- 9	-82	-78	+ 4	--	--	--	--	-45/ 150	-50/ 135
12	(5)	(4)	6.3	7.40	>150	50	<-22	+ 6	-59	-67	- 8	--	- .5	0	+2	- 0/ 145	-35/ 135
8	(4)	(5)	6.3	7.59	>150	27	<-22	+ 2	-85	-70	+15	+ .5	- .5	+2	+ .5	-26/ 87	0/ 66
8	(9)	(5)	6.3	7.59	>150	27	<-22	+ 2	-80	-70	+10	-1	- .5	+2	+ .5	+24/ 48	0/ 66
7	(3)	(4)	<6.6	7.21	>150	46	<-22	-7.5	-90	-50	+40	+7.5	- .5	-1.5	+1.0	-53/ 130	-25/ 130
4	(3)	(4)	6.32	7.70	>150	15.5	<-22	-2	-80	-50	+30	--	-1	--	+2	-50/ 130	-30/ 135
3	(4)	(2)	<6.6	7.52	>150	21	<-22	-3	+53	+60	+ 7	--	+1	+1.5	+ .5	- 4/ 98	0/ 105

KEY TO TABLE 3

K	=	Plasma potassium concentration (mEqu./L).
Δ K	=	Difference between test perfusion K and control K.
Type of Blood	=	H (Heparinised) : A.C.D. (Acid-Citrate-Dextrose).
B. E.	=	Base Excess (mEqu./L).
V. C. F.	=	% Change in V.C.F. after 60 seconds perfusion.
Δ V. C. F.	=	Algebraic Sum: test V.C.F. - minus control V.C.F.

TABLE 3

EFFECT OF HYPERKALAEMIA ON V.C.F.

Perfusion Pairs			Control K	Test K	Δ K	Type of Blood.	B. E.	Control V. C. F.	Test V. C. F.	Δ V. C. F.
Dog	Test	Cont.								
9	(3)	(2)	4.60	6.70	2.10	A.C.D.	-22	-44	+13	+57
9	(6)	(2)	4.60	7.00	2.40	A.C.D.	-22	-44	-80	-36
9	(10)	(9)	3.40	6.00	2.60	H.	-12.5	+18	-33	-51
15	(5)	(3)	3.30	6.50	3.20	A.C.D.	-22	-44	+18	+62
11	(9)	(7)	3.00	6.90	3.90	H.	- 6/-10	+55	+41	-14
11	(8)	(2)	3.10	7.15	4.05	A.C.D.	-22	+ 3	+14	+11
13	(8)	(3)	2.55	7.55	5.00	A.C.D.	-22	-60	-67	- 7
13	(6)	(1)	5.65	11.90	6.25	H.	- 3	-33	-45	-12
13	(7)	(1)	4.35	11.50	7.15	H.	- 3	-51	-50	+ 1
13	(9)	(3)	2.55	11.00	8.45	A.C.D.	-22	-60	-21	+39
15	(7)	(1)	5.65	14.45	8.80	H.	- 3	-33	-57	-24

KEY TO TABLE 4

T II	=	Change in T-wave amplitude (mm.) in Lead II of E.C.G. after 60 seconds perfusion.
Δ T II	=	TII (Test) -minus TII (Control).
Q-T	=	Change in Q-T interval (mm. at 2.5 cm/sec. paper speed) after 60 seconds perfusion.
Δ Q-T	=	Q-T (Test) minus Q-T (Control).
Rate	=	Change in pulse rate after 60 seconds/ Control pulse rate of that particular perfusion.
Δ Rate	=	% Change in rate (Test minus Control)
Alt.	=	Electrical alternans.
-	=	Record unsuitable for analysis.

TABLE 4

EFFECT OF HYPERKALAEMIA ON E.C.G.

Perfusion Pairs			Control T II	Test T II	Δ T II	Control Q-T	Test Q-T.	Δ Q - T	Control Rate	Test Rate	Δ Rate
Dog	Test	Cont.									
9	(3)	(2)	-1	+5	+6	+0.5	+0.5	0	-22 /106	+36 /84	+63 /100
9	(6)	(2)	-1	-4	-3	+0.5	+1.5	+1.0	-22 /106	-42 /120	-15 /100
9	(10)	(9)	+0.5	-2	-2.5	0	+1.5	+1.5	-22 /106	-25 /100	- 5 /100
15	(5)	(3)	+0.5	+3	+2.5	0	0	0	-12 /100	- 0 /115	+12 /100
11	(9)	(7)	Alt. +4 -1	+.5	Alt. -3.5 +1.5	+.5	+.5	+0	- 3 /128	+ 6 /130	+ 6 /100
11	(8)	(2)	Alt. +5.5 -0.5	0	Alt. -5.5 +0.5	--	+.5	--	- 5 /125	- 0 /130	+ 4 /100
13	(8)	(3)	-3	-4	-1	-5	0	+ .5	-10 /110	-15 /150	- 1 /100
13	(6)	(1)	+2	+3	+1	-.5	-.5	0	+ 5 /105	+25 /100	- 1 /100
15	(6)	(1)	-1.5	-2.5	-1.0	0	-1.0	-1.0	-14 /106	-31 /115	-14 /100
13	(7)	(1)	+2	-1	-3	-.5	-1.0	-.5	+ 5 /105	- 5 /135	+ 2 /100
13	(9)	(3)	-3	-1	+2	-.5	0	+.5	-10 /110	0 /160	+ 9 /100
15	(7)	(1)	-1.5	-1	+0.5	0	+.5	+.5	-14 /106	-37 /125	-16 /100

TABLE 5 EFFECT OF HYPOTHERMIA ON V.C.F.

PERFUSION			Type of Blood	Added Ca. per 50 ml.	Dog Wt. (Kg.)	% CH. V.C.F. 37°C	% CH. V.C.F. 10°	Δ V.C.F.
DOG	37°C	10°C						
13	1	2	H	-	20	-51	+ 8	+ 59
15	6	2	H	-	16.8	-45	+ 6	+ 51
15	1	2	H	-	16.8	-33	+ 6	+ 39
9	10	11	H	-	15.9	-33	+ 6	+ 39
14	1	2	H	-	15.5	-13	0	+ 13
Mean								+ 40.2
13	8	10	A.C.D.	.7	20	-67	+42	+109
13	9	10	A.C.D.	.7	20	-21	+42	+ 63
13	3	4	A.C.D.	.7	20	-60	-43	+ 17
15	9	8	A.C.D.	.4	16.8	-70	- 3	+ 67
15	5	4	A.C.D.	.7	16.8	+18	-31	- 13
15	3	4	A.C.D.	.7	16.8	-44	-31	+ 13
9	3	4	A.C.D.	.6	15.9	+13	0	- 13
9	5	4	A.C.D.	.6	15.9	-18	0	+ 18
9	6	8	A.C.D.	.6	15.9	-80	-85	- 5
14	3	4	A.C.D.	.7	15.5	-83	0	+ 83
Mean								+ 33.9

TABLE 6

Experiment.	Hep. Level u/ml.	Polybrene µG/ml.	Calcium to Polyb. delay	POLYBRENE CLOTTING TIME (Min.) Added 2% Calcium Chloride Solution/Ml. Blood							
				0	.01	.04	.08	.1	.2	.3	.4
1	2	30	0	9.5	8	9.5	10	-	13	-	28
			20	7.5	5	5	4	-	6.5	-	26
			40	6	9	6.5	7	-	7.5	-	12.5
			60	10	13.5	9.5	6	-	10.5	-	14
			24 hrs	11.5	6	9.5	7.5	0 (21hrs)	0 (4h)	0 (4h)	0 (4h)
2	2	30	0	8.5	9	8.5	12	13	16.5	17	50
			20	9	7	7.5	9	7	5.5	5.5	18.5
			60	9	11.5	7.5	7	6.5	8	9.5	11.5
			24 hrs	12	9	7	0	0	0	0	0
3	2	70	0	12.5	8	8.5	13	14.5	23	-	50
			20	12.5	8	7	8.5	11.5	7	-	50
			60	12	5	10.5	9	7	8.5	-	24
			24 hrs	14.5	7.5	11.5	9	0	0	-	0

TABLE 6 (Cont'd.)

Experiment.	HEP. LEVEL u./ml.	POLY-BRENE μ/ml.	CALCIUM TO POLYB. DELAY	POLYBRENE CLOTTING TIME (Min.) Added 2% Calcium Chloride Solution/Ml. Blood							
				0	.01	.04	.08	.1	.2	.3	.4
4	2	70	0	5.5	5	7	6	6	8	12	40
			24 hrs	13	8.5	6	8.5	0 (15h.)	0 (15h.)	0 (15h.)	0 (15h.)
5	5	60	0	11	10	12	14.5	14	14	21.5	24.5
			20	11	6.5	7	5.5	6.5	7.5	12	13
			60	11	6	6.5	5.5	7.5	10	14	16
			24hrs	8	6	4.5	5	4	5	5.5	4.5
6	5	60	0	7.5	8.5	9.5	10.5	11	14	20	28.5
			20	7.5	5	5	5	5	-	7.5	23
			60	8	3.5	3	4.5	5	7	8	11
			24hrs	7.5	5	5	6	6	6.5	10	12.5

TABLE 7

Experiment.	Heparin Level u/ml.	Polybrene µ/ml	Solutn. Added	POLYBRENE CLOTTING TIME mls. calcium chloride solution/ml. blood. 0	.01	.04	.08	.1	.2	.3	.4
7	2	30	$Ca.Cl_2.6H_2O$: 2%	11.5	6	9.5	0 (21h)	0 (4hr)	0 (4hr)	0 (4hr)	0 (4hr)
	2	30	Na.Cl 2%	11.5	11	11	11	11	16.5	28.5	60
8	2	30	$Ca.Cl_2.6H_2O$: 2%	10	9	8.5	0	0	0	0	0
	2	30	Na.Cl. 2%	10	10	10	12	11	18.5	33	50
9	2	30	$Ca.Cl_2.6H_2O$: 2%	8	5	6	4.5	0 (15hr)	0 (12h)	0 (12h)	0 (12h)
	2	30	Na.Cl 2%	8	6.5	8	8	7.5	13	27	50
10	5	60	$Ca.Cl_2.6H_2O$: 2%	8	-	-	5	4	5	5.5	4.5
	5	60	Na.Cl 2%	8	-	-	4	4	4	4.5	11

TABLE 8

Experiment	Base Excess mEq/l.	Heprn. Level u/ml.	Polybrene ug/ml.	POLYBRENE CLOTTING TIME mls. calcium chloride soln./ml. blood					
				0	.01	.04	.08	.2	.4
11	+ 1	2	30	3	3	5	4	4	12.5
	+10	2	30	3	4	4	3	3.5	23
	+18	2	30	5	3.5	3.5	6.5	13	30
12	0	2	30	3	5.5	3	2.5	5	38
	+10	2	30	4.5	2.5	2.5	2	3.5	3.5
	+18	2	30	5	5	3.5	3.5	6.5	0
13	+ 0	2	90	5					
	+ 8	2	90	5					
	+16	2	90	8.5					
14	+ 1	2	90	8					
	+10	2	90	7					
	+18	2	90	10					
15	+ 0	2	60	6.5					
	+ 8	2	60	6.5					
	+16	2	60	4.5					
16	+ 1	2	60	7.5					
	+10	2	60	6.5					
	+18	2	60	7.5					

EFFECT OF ALKALI ON POLYBRENE CLOTTING TIME.

TABLE 9

Experiment No.	Heprn Level units/ml.	PolyB. Level µg/Ml.	$Ca.Cl_2$ to PolyBr. delay	POLYBRENE CLOTTING TIME Mls. Calcium Chloride Solution 2% per Ml. A.C.D. Blood							
				0	0.01	0.04	0.08	0.1	0.2	0.3	0.4
17	0	0			160	160	90	26	23	20	23
18	2	30	0	—	—	—	130	33	18	25	26
			20	—	—	—	130	33	14	22	23
			1 hr.	—	—	—	128	31	12	16	20
			24 hrs.	—	—	—	120	56	0(24 hrs)	0(24 hrs)	17
19	5	60	0	—	—	—	120	56	35	33	45
			20	—	—	—	120	48	31	32	44
			1 hr.	—	—	—	120	45	30	31	44
			24 hrs.	—	—	—	108	35	26	24	27
20	5	60	0	—	—	—	120	54	32	33	45
			20	—	—	—	120	47	30	33	48
			1 hr.	—	—	—	120	43	30	31	40
			24 hrs.	—	—	—	112	36	27	25	29

KEY TO TABLE 10.

Hep. = Heparin only injected (300 units/Kg. body weight).

∞ = Greater than 120 minutes.

C = Calcium injection immediately after blood sample:
20% Calcium Chloride Solution B.P. :
For doses see text.

- = Sample lost.

Minutes after Heparin Injection →	Polybrene Clotting Time (10μ titration)								
	0	15'	30'	45'	60'	75'	90'	105'	120'
DOG I									
a) Heparin	13.5	∝	24.5	12	5	9	10.5	11.5	12
b) Ca. Cl_2 x 3	12	∝	23 c	12 c	7 c	10	2	4	6
c) Ca. Cl_2 x 3	11	∝	16.5 c	10 c	4 c	5	20	12	3
d) Ca. Cl_2 x 3	6.5 c	∝ c	24 c	12	6.5	7	4	9.5	6
DOG II									
e) Heparin	4	58	19.5	20	7	5	7.5	8	4
f) Ca. Cl_2 x 1	3.5 c	35	56.5	46.5	9	3	3.5	7	11
g) Ca. Cl_2 x 3	4.5 c	∝ c	21. c	4.5	11	9	3.5	5	3.5
DOG III									
h) Heparin	8	24.5	—	15	11	16	10	6	12
i) Ca. Cl_2 x 3	9 c	13.5 c	15.5 c	6.5	10.5	11.5	10.5	12.5	9
DOG IV									
j) Heparin	4	77	5	2.5	4.5 c	4.5	3	5	2.5
k) Ca. Cl_2 x 1	9 c	92	6	5	5	3.5	5.5	4.5	5.5
l) Ca. Cl_2 infusion	13 C	61.5	32	6	6.5	5	4.5	2.5	7 C

The Effect of Calcium on Anticoagulation by Heparin in Vivo.
Polybrene Clotting Times after Heparin Injection.

TABLE 10

Minutes after Heparin Injection →	Thrombin Time (Sec.) 0	30'	45'	60'	75'	90'	105'	120'
DOG I								
a) Heparin	15	72	49	43	30	23	-	20
b) Ca. Cl_2 x 3	13	45	30 C	22 C	23 C	13	14	13
c) Ca. Cl_2 x 3	13	> 7 min	53 C	25 C	21 C	19	19	18
d) Ca. Cl_2 x 3	Not done							
DOG II								
e) Heparin	16	47	19	20	15	17	20	14
f) Ca. Cl_2 x 1	10 C	24	25	18	14	16	--	10
g) Ca. Cl_2 x 3	9 C	19 C	15 C	14.5	10	10	7.5	10
DOG III								
h) Heparin	10	22	18	15	14	14	12	10
i) Ca. Cl_2 x 3	12 C	65 C	45 C	41	30	28	23	22
DOG IV								
j) Ca. Cl_2								
k) Ca. Cl_2 x 1	NOT DONE							
l) Ca. Cl_2								

The Effect of Calcium on Anticoagulation by Heparin in Vivo.
Thrombin Clotting Times after Heparin Injection

TABLE 11

Expt.	Dog Wt. (Kg)	Av. Perf. Rate ml/min	Type of Blood	ml. Added Ca.	V. C. F.										B. P.			Comment
					Pre.	0	1'	3'	5'	10'	15'	20'	De. Can.	Post	Pre. Perf.	Post Perf.	1 hr Post	
1	18	960	A.C.D.	9	14	12	13	13	13	12	12	10	13	11	140	100	110	No ECG changes Died 18 hours after perfusion
2	22.2	2000	A.C.D.	9	16	15	24	20	17	17	18	17	16	15	104	120	124	Survivor
3	23	2200	A.C.D.	9	7	6	14	--	12	9	7	7	11	10	120	140	120	Increased R-volt during perfusio Survived.
4	28	2560	A.C.D.	9	9	8	11	10	10	10	8	9	11	12	112	130	130	T-wave depressi for 2 minutes. Survivor.
5	12.2	1050	A.C.D.	9	7	8	7	7	7	--	5	9	13	8	80	130	120	Anaesthetic ove dose. Gross EC abnormalities. Died 1 hr after perfusion.
6	21	1900	A.C.D.	9	Electrical fault in V. C. F. Channel									-	144	112	140	Survivor.
Mean (Expt. 1-4)			A.C.D.	9	11.5	10.2	15.5	14.3	13	12	11.3	10.8	12.8	11.5				
% Change in Mean (Expt. 1-4)					+11	0	+51	+40	+27	+11	+10	+ 5	+29	+17				

NOTE: Data from experiment 5 not included in calculations of percentage change in means.

TABLE 12.

Expt.	Dog Wt. (KG)	Av. Perf. Rate ml/min	Type of Blood	ml. Added Ca.	V. C. F. Pre.	0	1'	3'	5'	10'	15'	20'	Re. Can	Post	B. P. Pre. Perf.	Post Perf.	1 hr Post	Comment
7	19.7	2115	A.C.D.	6	6	6	7	7	7	8	8	8	10	9	100	80	116	Survivor: ? External Strab. first 6 hours
8	20.8	2440	A.C.D.	6	5	5	5	7	7	7	4	3	4	5	136	130	144	Early survivor. Found dead after 24 hours.
9	12	1200	A.C.D.	6	8	8	9	9	7	8	8	7	10	10	100	110	130	Survivor.
10	17.5	2120	A.C.D.	6	Electrical fault in V. C. F. Channel										120	120	180	Survivor
Mean (Expt. 7-9)			A.C.D.	6	6.3	6.3	7	7.7	7	7.7	6.7	6	8	8				
% Change in Mean (Expt. 7-9					0	0	+11	+21	+11	+21	+5	-5	+25	+25				
11	18.5	1650	H	0	8	8	10	10	7	7	8	8	8	8	112	120	120	Survivor.
12	19	1800	H	0	10	10	15	17	16	10	10	10	10	11	120	110	130	Survivor.
13	20	1440	H	0	8	9	13	10	9	9	9	9	8.5	8	120	112	100	Survivor.
Mean (Expt. 11-13)			H	0	8.7	9	12.7	12.3	10.7	8.7	9	9	8.8	9				
% Change in Mean (Expt. 11-13)					-3	0	+41	+37	+22	-3	0	0	-2	0				

TABLE 12 (Continued)

KEY TO TABLE 13

pH_B	=	pH corrected (Rosenthal factor) to body temperature.
K	=	Plasma Potassium (mEqu./L).
Na	=	Plasma Sodium (mEqu./L).
Buffer Base	=	Plasma Buffer Base (mEqu./L).
Hb.	=	Plasma Haemoglobin (mgm. per 100 ml.).
Prime	=	"Converted" A.C.D. blood used to prime oxygenator.
Cont.	=	Arterial blood sample prior to cardiac bypass.

TABLE 13

ELECTROLYTE CHANGES DURING CARDIO-PULMONARY BYPASS IN DOGS

	pH_B							K						Na			
Expt.	Prime	Cont.	Perfusion 4'	Perfusion 20'	Post Perfusion 1 hr	Post Perfusion 2 hr	Post Perfusion 3 hr	Prime	Cont.	Perf. 20'	Post Perfusion 1 hr	Post Perfusion 2 hr	Post Perfusion 3 hr	Prime	Cont.	Perf 20'	Post 3hrs
7	7.00	7.40	7.15	7.33	7.40	7.38	7.47	3.6	2.6	2.45	2.70	3.0	3.2	146	150	154	162
8	7.08	7.31	7.02	7.08	7.20	7.20	7.25	2.6	3.8	3.1	3.7	3.5	3.6	154	162	158	154
9	7.00	7.31	7.06	7.27	7.35	6.99	7.24	3.2	3.2	3.1	3.4	3.5	3.9	160	156	156	150
10	7.00	7.57	7.28	7.38	7.02	7.30	7.40	3.0	2.50	2.3	2.2	2.6	3.3	156	154	146	154
Mean	7.02	7.40	7.13	7.26	7.24	7.22	7.28	3.10	3.03	2.74	3.0	3.15	3.50	154	155	153	155
	Buffer Base							Haematocrit						Hb.			
7	23	38	25	36	40	40	44	34	45	45	41	37	41	34	18.8	320	196
8	21	37	23.5	25	29	31	34	35	59	42	44	45	44	363	12.1	320	120
9	23	34	24	33	37	23	34	36	47	43	43	43	44	17.5	6.7	66	54
10	24	58	32	37	22	32	40	34	58	41	41	44	43	10	52	3160	1338
Mean	23	42	26	33	32	32	38	35	52	43	42	42	43				

KEY TO TABLE 14

Monitored data on A.C.D. Case I
Gilbert Young. Age 33. Wt. 63 Kg.

Spec.	-	Arterial blood specimen
Temp. °C	-	Oseophageal temperature
pH_{38}	-	Arterial blood pH measured at 38°C
pH_B	-	Arterial blood pH corrected to body temperature
$PCO_{2_{38}}$	-	Arterial blood PCO_2 measured at 38°C
PCO_{2_B}	-	Arterial blood PCO_2 corrected to blood temperature
Sta. Bic.	-	Standard bicarbonate (mEqu./l.)
K^+, Na^+, Cl.	-	Plasma potassium, sodium, chloride (mEqu./l.)
Haem.	-	Plasma haemoglobin (mg. per cent)
Hct.	-	Arterial blood haematocrit
W.B.C.	-	White blood count (cells per cu. mm.)
Plat. X1000	-	Platelet count x1000/cu. mm.
B.P. Syst.	-	Systemic Blood Pressure
C.T.	-	Clotting time.

TIME	SPEC.	Temp °C.	pH38	pHB	Pco_2 38	Pco_2 B	STA. BIC.	K^+	Na^+	Cl^-	Haem.	Hct.	W.B.C.	PLAT. ×1000	B.P. SYST.	C.T.	Remarks
15.3.65 16.50	A	37	7.33	7.34	43	41	21.5	4.2	142	106	0	43	7,950	159	140	-	18 hours pre-op.
16.2.65 09.35	1	37.5	7.53	7.54	25	25	22.5	4.13	142.3	111	0	43	6,000	118	150	5	Anaesthesia Induced
11.16	2	34	6.92	6.98	36	31	7.3	4.91	150	87	0	37	6,000	159	---	-	A.C.D. Blood (Oxygenator)
11.32															130	-	Start Bypass
11.34	3	35	7.32	7.38	24	21	11.1	3.87	143.4	100	0	41.5	5,700	179	100	-	2 min. "in parallel
11.37	4	35	7.35	7.40	23	20	14.9	3.31	144.4	109	28.2	39	--	-	95	-	Total Bypass
11.42	5	35	7.37	7.42	23	20	15.0	3.20	144	109	30	40	--	-	80	-	10 min. Perfusion
11.47	6	35	7.32	7.37	28	25	15.2	-	-	-	-	-	--	-	60	-	15 min. Perfusion Defibrillation
12.00	7	35	7.32	7.37	31	27	16.2	3.16	143.4	110	40.3	40	7,225	129	90	-	End Bypass
12.30	8	36	7.61	7.64	14	13	17.4	3.23	140.7	111	-	41.5	9,900	147	130	6	
13.00	9	37	7.24	7.33	53	51	20.4	3.08	141.7	109	-	43	27,175	155	---	-	
1.400	10	37	7.34	7.36	38	36	20.0	3.76	140.3	110	-	44	5,200 Clumps	164	---	-	
16.00	11	38.4	7.34	7.34	43	44	22	4.41	148	109	-	44	22,825	152	---	-	
17.00	12	38	7.31	7.31	43	43	10.5	4.70	154	-	0	43.5	24,150	111	---	-	
17.3.65 12.00	13	37	7.28	7.30	45	43	20.0	4.70	154	103	-	39.5	11,700	74.4	120	6	24hrs. Post-Op.

TABLE 14

HUMAN A.C.D. BLOOD PERFUSION : CASE I : BIOCHEMICAL DATA.

TABLE 15

DOGS USED FOR CORONARY PERFUSION

DOG NO.	WEIGHT (Kg.)
1	6.3
2	11.4
3	10.9
4	15
5	19
6	19
7	15
8	14.5
9	15.9
10	13.2
11	15.5
12	10.9
13	20
14	15.5
15	16.8

TABLE 16

PERFUSION	TYPE OF BLOOD	TEMP.	ADDED Ca.	pH	PCO_2	STA. BIC.	BASE EXCESS	K	Na	Ca	V.C.F.						B.P.	
											CONT.	30"	60"	% CH. 30"	% CH. 60"	REC.	CONT.	REC.
DOG I	---	37	--	7.25	59	22.5	-0.5	4.8	146	4.8								
1 (1)	H	37	0	7.30	50	21.5	0	4.5	145	4.8	20	20 30	26	+ 25	+ 30	14	86	80
1 (2)	ACD	37	.6	6.35	>150	< 6	<-22	3.0	150	25.1	15	3	3	- 80	- 80	9 14	83	70
1 (3)	ACD	37	.9	6.35	150	< 6	<-22	3.0	150	42.0	17	40	40	+130	+130	18	75	70
1 (4)	ACD	37	.7	6.35	150	<6	<-22	3.10	150	31.2	18	40	40	+120	+120	22	80	75
1 (5)	ACD	37	.6	6.60	150	8.6	<-22	3.0	158	25.0	21	25 35	20	+ 48	- 5	21	85	70
1 (6)	ACD	37	.6	6.35	150	< 6	<-22	3.1	150	26.1	20	30 10	E.S.	0	---	19	75	70
1 (7)	ACD	37	.6	6.35	150	< 6	<-22	3.0	153	24.5	19	10	7	- 42	- 63	22	75	70
1 (8)	H	37	0	7.30	50	21.5	0	4.5	145	4.8	18	24 30	36	+ 50	+100	22	70	75
1 (9)	ACD	37	.6	6.35	150	< 6	<-22	3.0	147	25.0	18	16 40	5 10	+ 56	- 61	18	70	65
1 (10)	ACD	37	.4	6.35	150	< 6	<-22	3.0	150	18.5	17	10 15	3	- 26	- 71	14	70	65
1 (11)	H	37	0	7.30	50	21.5	0	4.5	145	4.8	16	40	40	+150	+150	30	65	50
1 (12)	ACD	37	0	6.35	150	< 6	<-22	3.2	153	5.0	18	5	5	- 72	- 72	5	65	0

TABLE 17

PERFUSION	TYPE OF BLOOD	T-WAVE					Q-T INTERVAL					RATE				
		CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"	CONT	30"	60"	CH. 30"	CH. 60".
1 DOG I	---															
1 (1)	H	-7	-7	-7	-0	-0	10	--	--	--	—	115	105	100	-10	-15
1 (2)	ACD	-7	-9	-9	-2	-2	10.5	15	14	+4.5	+3.5	120	110	110	-10	-10
1 (3)	ACD	-8	-2.5	-3	+5.5	+5	11	10	10	-1	-1	120	104	94	-16	-26
1 (4)	ACD	-7	-4	-4	+3	+3	11	11	11	0	0	125	115	115	-10	-10
1 (5)	ACD	-8	-9	-	-1	-	11	11	11.5	0	+5	115	115	115	0	0
1 (6)	ACD	7	-	-	-	-	11	12	-	+1	-	115	105	110	-10	- 5
1 (7)	ACD	8	-	-	-	-	11	13	12	+3	+2	120	110	110	-10	-10
1 (8)	H	-4.5	-3	-4.5	+1.5	0	12	12	12	0	0	120	115	104	- 5	-16
1 (9)	ACD	-6	-14	E.S.	-8	-	11	15	15	+4	+4	100	94	100	- 6	0
1 (10)	ACD	-6	E.S.	E.S.	-	-	11	14	15	+3	+4	100	76	84	-24	-16
1 (11)	H	-6	-3	-3	+3	+3	12	12	12	0	0	84	90	92	+ 6	+ 8
1 (12)	ACD	2	E.S.	E.S.	-	-	12	E.S.	E.S.	-	-	80	65	52	-15	-28

TABLE 18

PERFUSION		TYPE OF BLOOD	TEMP.	ADDED Ca.	pH	PCO_2	STA. BIC.	BASE EXCESS	K	Na	Ca	V.C.F.						B.P.	
												CONT.	30"	60"	%CH. 30"	%CH. 60"	REC.	CONT.	REC
2	DOG 1	---	37	--	7.44	21	19.0	-6	2.2	152.5	7.4								
	2 (1)	H	37	0	7.07	105	21.6	-1.8	4.9	145	3.5	13.5	9	9	-33	-33	9	75	70
	2 (2)	ACD	37	.6	6.73	>150	21.6	-1.5	3.4	156	25.6	10	4	1.5	-60	-85	14	70	78
	DOG 2	---	37	--	7.52	23	21.6	-1.5	---	---	---								
	2 (3)	ACD	37	.6	6.6	>150	<6	<-22	3.4	156	25.6	10	3	1.5	-70	-85	14	70	84
	2 (4)	ACD	37	.8	6.6	>150	<6	<-22	3.4	156	36.6	12	5.5	4	-54	-67	15	80	90
	2 (5)	ACD	37	.8	6.75	>150	21.6	-1.5	3.4	175	32.6	11	3	2	-73	-82	20	60	85
	DOG 3	---	32°C	--	7.41	24	17.5	-7.2	3.1	150	13.2								
	2 (6)	ACD	37	1.0	6.6	>150	<6	-22	3.4	156	40	10	8	6	-20	- 4	13	60	65
	2 (7)	H	37	0	7.07	105	21.6	-1.8	4.9	145	3.47	9	4	3	-56	-67	5.5	60	60
3	DOG I	---	37	--	7.31	33	17.8	-8.8	3.6	156	4.5								
	DOG II	---	37	--	7.36	34	19.5	-5.3	3.6	156	4.5								
	3 (1)	H	37	0	7.24	40	19.0	-6.2	4	140	5	19	24	17	+26	-10	12	120	100

PERFUSION		TYPE OF BLOOD	T - WAVE					Q-T INTERVAL					RATE				
			CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"
2	DOG 1																
	2 (1)	H	-1	-1	-1	0	0	7	7	7	0	0	120	110	105	-10	-15
	2 (2)	ACD	1	-	-	-	-	8	8.5	10	+5	+2	115	94	74	-21	-41
	DOG 2																
	2 (3)	ACD	-1.5	-1.5	-2.5	0	-1	8	8	9	0	+1	120	94	80	-26	-40
	2 (4)	ACD	-1	-1	-1	0	0	7.5	8	9	+.5	+1.5	120	98	88	-22	-32
	2 (5)	ACD	1	1.5	1	.5	0	8.5	8.5	-	0	5.5	110	75	58	-35	-52
	DOG 3																
	2 (6)	ACD	-1	-1	-1	0	0	8	8.5	9	+.5	+1	110	100	98	-10	-12
	2 (7)	H	-1	-1	-1	0	0	8.5	8	8	-.5	-.5	106	96	96	-10	-10
3	DOG I	---															
	DOG II	---															
	3 (1)	H	-1	+2	+1	+3	+2	7	7	7	0	0	118	115	98	- 3	-20

TABLE 20

	Perfusion	Type of Blood	Temp	Added Ca.	pH.	PCO_2	Sta. Bicarb.	B.E.	K	Na	Ca.	VCF Cont	VCF 30"	VCF 60"	VCF % Ch. 30"	VCF % Ch 60"	VCF Rec.	B.P. Cont.	B.P. Rec.
3	3 (2)	ACD	37	.6	7.52	21	21.5	- 3	4	180	26	12	17	19	+42	+60	13	100	86
	3 (3)	ACD	37	.6	6.81	170	26	+2.1	4	180	26.5	13	12	9	- 8	-30	15	84	90
	3 (4)	ACD	37	.6	<6.6	>150	< 6	<-20	4	145	28	15	9	23	-40	+53	16	88	90
	DOG III	---	37	--	7.20	27.6	12.6	-1.6	3.8	141	10								
	3 (5)	ACD	37	.4	<6.6	>150	< 6	-20	4	145	10	21	27	24	+29	+14	21	80	84
	DOG IV	---	37	--	7.26	51	+ 9.5	<- 6	--	---	--								
	3 (6)	H	37	0	7.26	51	19.5	- 6	4.0	140	4.5	16	24	30	+50	+88	20	70	80
	3 (7)	ACD	37	0	<6.6	>150	6	<-22	4	145	5.0	16	5	1	-69	-94	1	80	20
	3 (8)	ACD	37	.2	6.4	>150	6	<-22	4	147	12	4	E.S. 4	2	0	-50	4.5	70	45
4	DOG I	---	37	--	7.31	32.5	16	- 8	4.8	140	5.05								
	4 (1)	H	37	0	7.34	50.5	19	- 3	4.0	142.5	7.45	11	13	13	+18	+18	11	100	120
	DOG II	---	37	--	7.42	35	22.5	-0.5	4.8	140	5.05								
	4 (2)	ACD	37	.6	6.32	>150	6	<-22	2.7	160	26	10	10.5	10	+15	0	10	100	100

TABLE 21

PERFUSION		TYPE OF BLOOD	T - WAVE					Q-T INTERVAL					RATE				
			CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH 60"	CONT	30"	60"	CH. 30"	CH 60"
3	3 (2)	ACD	-1	-1	0	0	+1	7.5	8	8	+5	+.5	105	105	105	0	0
	3 (3)	ACD	-1	--	-6	-	-5	7.5	8.5	10	+1.0	+2.5	100	90	90	-10	-10
	3 (4)	ACD	-2	--	--	--	--	8	9	9.5	+1.0	+1.5	98	88	94	-10	- 4
	DOG III																
	3 (5)	ACD	-2	--	--	--	--	8	8.5	8.5	+.5	+.5	105	100	100	- 5	- 5
	DOG IV																
	3 (6)	H	-5	--	--	--	--	8.5	8.5	8.5	0	0	98	98	98	0	0
	3 (7)	ACD	-4	-15	-12	-11	-8	8	11	13	+ 3	+ 5	95	90	75	- 5	-20
	3 (8)	ACD	-4	- 9	-11	- 5	-7	8	11	12	+ 3	+ 4	90	85	75	- 5	-15
4	DOG I	---															
	4 (1)	H	-4	- 3	- 3	+ 1	+1	8	8	6	0	- 2	130	135	140	+ 5	+10
	DOG II																
	4 (2)	ACD	-3.5	-3.5	- 3	- 0	+.5	8	8	7	0	- 1	130	130	125	0	- 5

TABLE 22

PERFUSION		TYPE OF BLOOD	TEMP.	ADDED Ca.	pH	PCO_2	STA. BICARB.	B.E.	K	Na	Ca.	V.C.F. CONT	V.C.F. 30"	V.C.F. 60"	V.C.F. %CH. 30"	V.C.F. %CH. 60"	V.C.F. REC	B.P. CONT	B.P. REC
4	4 (3)	ACD	37	.4	6.32	>150	< 6	<-22	2.7	160	22	10	3	2	- 70	- 80	12	100	100
	4 (4)	ACD	37	.4	7.70	15.5	21	- 2	--	>180	22	10	5	5	- 50	- 50	.9	100	100
	4 (5)	ACD	37	.4	6.91	114	16	- 9	2.7	>180	22	9	3.5	2	- 61	- 78	8	100	110
	DOG III		37		7.46	28	17.5	- 5.5	2.7	142.5	6.8								
	4 (6)	ACD	37	.4	6.76	45	< 6	<-22	2.7	160	22	8	3.5	2.5	- 56	- 56	8	100	100
	4 (7)	ACD	37	.4	7.54	110	54	>+30	4	>200	22	8.5	6	5	- 30	- 40	13	90	110
	4 (8)	H	37	0	7.34	50.5	19	- 3	4.0	142.5	7.5	11	8.5	10	- 23	- 9	9	100	100
	4 (9)	ACD	37	.4	6.3	>150	< 6	<-22	2.7	160	22	8.5	4	1.5	- 53	- 82	10	100	100
	4 (10)	ACD	37	0	7.33	110	>60	+30	2.7	250	5.2	7	4.5	.5	- 80	- 93	7	100	120
	DOG IV		37						2.6	147.5	7.2								
	4 (11)	ACD	20	1.2	6.6	>150	< 6	<-22	2.7	160	48	9	12	11	+ 33	+ 22	8	90	110
5	DOG I		37		7.37	32	19.2	- 5	3.2	137.5	5.4								
	5 (1)	ACD	37	0	6.3	160	<-6	<-22	2.4	155	5.0	19	0	0	-100	-100	19	100	8

TABLE 23

PERFUSION		TYPE OF BLOOD	T-WAVE					Q-T INTERVAL					RATE				
			CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"	CONT	30"	60"	CH. 30"	CH. 60"
4	4 (3)	ACD	-3	-2	--	+ 1	--	8	9	--	+1	--	130	82	80	- 48	-50
	4 (4)	ACD	- 2	-2	-3	0	- 1	8	8.5	10	+.5	+2	135	105	105	- 30	-30
	4 (5)	ACD	-2	E.S.	E.S.	----	----	8	9	-	+1	--	130	80	80	- 50	-50
	DOG III	---															
	4 (6)	ACD	-3	E.S.	E.S.	--	--	7.5	--	--	--	--	125	100	94	- 25	-31
	4 (7)	ACD	-1.5	-4	-4	-2.5	-2.5	7	8	8	+1	+1	140	136	140	- 4	0
	4 (8)	H	+1	+1	--	0	--	6	--	--	--	--	140	100	100	- 40	-40
	4 (9)	ACD	-2	-2	--	0	--	6	--	--	--	--	150	115	105	- 35	-45
	4 (10)	ACD	-3	-3	E.S.	0	--	6	--	--	--	--	140	100	100	- 40	-40
	DOG IV	---															
	4 (11)	ACD	-2	E.S.	E.S.	--	--	6	E.S.	E.S.	--	--	150	95	92	- 55	-58
5	DOG I	---															
	5 (1)	ACD	-5	-4	-5	+1	0	6	9	10	+3	+4	140	120	110	- 20	-30

TABLE 24

PERFUSION		TYPE OF BLOOD	TEMP.	ADD. Ca.	pH	PCO_2	STA. BIC.	B.E.	K	Na	Ca.	V.C.F. CONT	30"	60"	% CH. 30"	% CH. 60"	REC.	B.P. CONT.	REC.
5	5 (2)	H	37	0	7.29	44	20.5	-39	3.45	140	5.6	18	9.5	10	- 47	- 44	18	90	92
	5 (3)	ACD	37	.6	6.3	>150	< 6	<-22	2.4	155	26.0	18	6	5.5	-67	-70	22	84	100
	5 (4)	ACD	37	.6	6.6	92	<6	<-22	2.4	155	26.0	16	5	5	- 69	-69	16	68	80
	5 (5)	ACD	37	.6	7.04	61	13.4	-15	2.4	155	26.0	13	5	3.5	- 62	-73	9	60	80
	DOG II	---	36	--	7.21	72	24.2	+1.9											
	5 (6)	H	37	0	7.13	92	20	-4.5	3.45	145	5.6	10	7	7	- 30	-30	10	64	68
	5 (7)	ACD	37	.6	7.09	200	51	+30	--	--	26.0	8	3.5	3	- 56	-62	10	30	40
	DOG III	---	31		7.00	65	13.6	-12	5.60	147.5	5.4								
	5 (8)	ACD	20	1.0	6.3	160	<6	<-22	2.4	155	41	7	8	8	+ 14	+14	10	40	40
	5 (9)	H	37	0	7.00	65	13.6	-12	5.6	147.5	5.4	6	7	8.5	+ 16	+40	6	20	36
6	DOG I		37.5		7.40	30	19.5	-4.2	3.2	137.5	6.6								
	6 (1)	H	37	0	7.00	120	18	-7	5.1	135	6.1	19	20	19	+ 5	0	14	92	76
	6 (2)	ACD	37	.6	6.35	>150	6	<-22	3.4	147.5	22	16	12	8	- 25	-50	18	92	100

TABLE 25

PERFUSION	TYPE OF BLOOD	T-WAVE					Q-T INTERVAL					RATE				
		CONT.	30"	60"	CH. 30"	CH. 60"	CONT	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"
5	5 (2)	H	-9	-10	-10.5	-1	-1.5	6	7	7	+1	+1	135	122	125	- 13	- 10
	5 (3)	ACD	-9	- 9	-10	0	-1	6	7	8	+1	+2	150	130	116	- 20	- 34
	5 (4)	ACD	-9	-10	-11	-1	-2	6	7	8	+1	+2	150	120	120	- 30	- 30
	5 (5)	ACD	-8	-10	-11	-2	-3	6	7	8	+1	+2	150	125	106	- 25	- 44
	DOG II	---															
	5 (6)	H	-9	-10	-10	-1	-1	6.5	7	7	+ .5	+ .5	150	112	102	- 38	- 48
	5 (7)	ACD	-12	-11	-11	+1	+1	7.5	9	11	+1.5	+3.5	86	84	84	- 2	- 2
	DOG III																
	5 (8)	ACD	-7	-5.5	- 5	+1.5	+2	8	9.5	11	+1.5	+3	67	58	53	- 9	- 14
	5 (9)	H	-6	-6	- 6	0	0	8	9	9	+1	+1	65	55	57	- 10	- 8
6	DOG I																
	6 (1)	H	-1	+1	0	+2	+1	8	8	8.5	0	+ .5	105	90	88	- 15	- 17
	6 (2)	ACD	-1.5	-3	- 4	-1.5	-2.5	9	10	12	+1	+3	96	88	74	- 8	- 22

TABLE 26

PERFUSION		TYPE OF BLOOD	TEMP.	ADDED Ca	pH.	PCO_2	STA. BIC.	B.E.	K	Na	Ca.	V.C.F						B.P.	
												CONT.	30"	60"	%CH. 30"	%CH. 60"	REC.	CONT.	REC.
6	6 (3)	ACD	37	0	7.34	46.5	24.2	+1.5	3.4	147.5	5.1	20	6	3	-70	-85	20	110	110
	6 (4)	ACD	37	.6	6.5	46	6	<-22	3.4	147.5	22	20	16	9	-20	-55	10	90	84
	DOG II		35		7.32	30	16	-8.7	3.2	137.5	6.7								
	6 (5)	ACD	37	.6	7.30	46	20.5	-3.5	(spec Lost)		)	22	22	0	0	-100	0	88	0
	DOG III		37		7.59	23.5	26.5	+5	3.2	137.5	6.7	0							
7	DOG I		37		7.31	32	16.5	-9	3.1	150	4.7								
	DOG II		37		7.43	31	21.7	-1.5	3.9	152.5	—								
	7 (1)	H	37	0	7.29	46	20.5	-3.5	4.6	137.5	5.2	10	13	13.5	+30	+ 35	11.5	100	100
	7 (2)	ACD	37	.6	6.6	>150	< 6	<-22	3.0	150	26	10.5	10.5	9.5	0	- 15	10	100	110
	7 (3)	ACD	37	.4	6.6	>150	< 6	<-22	3.0	150	22	10.5	2	1.5	-80	- 90	12	110	—
	7 (4)	ACD	37	.4	7.21	46	17	-7.5	2.8	190	22	10	5	5	-50	- 50	8	—	—
	7 (5)	ACD	37	.4	6.6	>150	16.7	-8	—	—	—	9	3	2	-55	- 78	8	100	100
	DOG III		35		7.37	26	17	-7.5	3.4	142.5	4.9								

TABLE 27

PERFUSION	TYPE OF BLOOD	T-WAVE.					Q-T INTERVAL.					RATE				
		CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"	CONT.	30"	60"	CH. 30"	CH. 60"
6	6 (3)	ACD	-2	-5	- 5	-3	-3	10	13	14	+3	+4	100	72	74	-28	-26
	6 (4)	ACD	-2	-1.5	-1.5	+.5	+.5	9.5	9.5	10	0	+.5	96	94	80	- 2	-16
	DOG II																
	6 (5)	ACD	-2	-1.5	V.F.	+.5	V.F.	10	10	V.F.	0	—	96	88	—	- 8	—
	DOG III																
7	DOG I																
	DOG II																
	7 (1)	H	-4	-3.5	-3.5	+.5	+.5	6	6	6	0	0	130	135	130	+ 5	0
	7 (2)	ACD	-3	-3	-2.5	0	+.5	6	5.5	6	-.5	0	135	130	126	- 5	- 9
	7 (3)	ACD	-2.5	E.S. -3	E.S. +5	-.5	+7.5	6	E.S. 7	E.S. 7	+1	+1	130	77	78	-53	-52
	7 (4)	ACD	-2.5	-2.5	-3	0	-.5	6	6	7	0	+1	130	105	105	-25	-25
	7 (5)	ACD	-2.5	E.S. +6	E.S +6	+8.5	+8.5	6	E.S. 7	E.S. 8	+1	+2	126	80	80	-46	-46
	DOG III																

TABLE 28

PERFUSION		Type of Blood	TEMP.	Add Ca	pH	P_{CO_2}	STA. BIC	B.E.	K	Na	Ca	V. C. F. CONT.	30"	60"	%CH 30"	%CH 60"	REC.	B. P. CONT.	REC
7	7 (6)	ACD	37	.4	6.6	40	6	-22	--	--	--	- 8	3.5	2	-55	-75	8	90	115
	DOG IV.		35		7.47	33	24	+1	3.4	155	-								
	7 (7)	ACD	37	.4	7.40	150	55	+30	-	-	-	8	5.5	5.5	-31	-31	13	100	110
	7 (8)	H	37	0	7.29	46	20.5	-3.5	4.6	137.5	5.2	10	8	9	-20	-10	9.5	110	115
	7 (9)	ACD	37	.4	6.6	150	6	-22	3.0	150	22	8.5	4	2	-53	-76	10	110	130
	7 (10)	ACD	37	0	7.45	150	55	+30	3.0	220	5.00	9	1.5	.5	-83	-95	9	110	130
	7 (11)	ACD	37	1.2	6.6	150	6	-22	3.0	150	48	9	12.5	11	+38	+22	9	-	110
8	DOG I		37		7.24	28	13.8	-13	3.2	137.5	5								
	DOG II		37		7.49	28	25	+3.5	3.2	137.5	5								
	8 (1)	H	37	0	7.35	26	16	-9.9	4.18	137.5	6.5	13	10	9	-23	-31	10	80	84
	8 (2)	ACD	37	.6	6.3	150	6	-22	2.53	142.5	26.3	6	5	5	-17	-17	7	40	40
	8 (3)	ACD	37	.5	6.3	150	6	-22	2.53	142.5	29	10	8	5	-20	-50	13	24	32
	DOG III		37						3.00	150.0	8.2								

TABLE 29

Perfusion		Type of Blood	T-Wave					Q-T Interval					Rate				
			Cont.	30"	60"	Ch. 30"	Ch. 60"	Cont	30"	60"	Ch. 30"	Ch. 60"	Cont	30"	60"	Ch. 30"	Ch. 60"
7	7 (6)	ACD	-3	E.S.	E.S.	--	---	6	7	E.S.	+1	---	130	100	100	- 30	- 30
	DOG IV.																
	7 (7)	ACD	-2	-3	--	- 1	--	6	6	--	0	--	140	135	140	- 5	0
	7 (8)	H	+1	+3	--	+ 2	--	6	--	--	--	--	140	115	100	- 25	- 40
	7 (9)	ACD	+1	--	--	--	--	6	--	--	--	--	150	120	125	- 30	- 25
	7 (10)	ACD	-3	--	E.S. +3	--	+ 6	6	--	11	--	+5	145	105	115	- 40	- 30
	7 (11)	ACD	-2	E.S.	E.S. +5		+ 7	5	--	E.S. 8	--	+3	145	95	100	- 50	- 45
8	DOG I																
	DOG II																
	8 (1)	H	-5	4.5	4.5	+.5	+.5	8	9	8	+1	0	110	100	100	- 10	- 10
	8 (2)	ACD	-5	-5	-6	0	-1	10	11	12	+1	+2	86	75	82	- 11	- 4
	8 (3)	ACD	-2.5	-2.5	-4.5	0	-2.5	10	11	13	+1	+3	92	80	70	- 12	-22
	DOG III																

TABLE 30

Perfusion		Type of Blood	Temp.	Added Ca.	pH	PCO_2	Sta. Bic.	B.E	K	Na	Ca.	V.C.F. Cont.	V.C.F. 30"	V.C.F. 60"	V.C.F. % Ch 30"	V.C.F. % Ch 60"	V.C.F. Rec.	B.P. Cont.	B.P. Rec.
8	8 (4)	ACD	37	.4	6.3	>150	<6	<-22	2.53	142.5	19	13	5	2	-62	-85	13	48	60
	8 (5)	ACD	37	.4	7.59	27	25.4	+ 8	2.5	170+	19	13	13	11.5	0	-11	13.5	46	52
	8 (6)	ACD	37	.4	6.88	>150	24.0	+ 2	2.53	170+	19	16.5	10	5	-39	-70	22	48	52
	DOG IV		37		7.16	53	16	- 9.5	3.00	147.5	7.6								
	8 (7)	ACD	37	.4	6.6	16	<6	<-22	2.53	142.5	19	21	20↓ 13↑	14	-19	-32	17	52	48
	8 (8)	ACD	37	.4	7.42	105	42.5	+25	2.50	200	19	16	10	8	-38	-50	16	48	48
	8 (9)	ACD	37	.4	6.3	>150	<6	<-22	2.53	142.5	19	20	9	4	-55	-80	15	50	52
	DOG V		30		7.17	33	11.2	-17.8	4.50	140	9.9								
	8 (10)	H	37	0	7.35	26	16	- 9.9	4.18	137.5	6.5	19	18	17	- 5	-11	17	50	30
	8 (11)	H	37	0	7.08	38	11.2	-17.8	4.18	137.5	9.89	17	22	25	+29	+47	--	40	40
9	DOG I	-	37	-	7.40	25.5	18.5	- 6.5	3.85	150	4.15								
	9 (1)	H	37	-	7.40	25.5	18.5	- 6.5	3.85	150	4.15	13	12	11	- 8	-16	10.5	80	60
	9 (2)	ACD	37	.6	6.3	>150	<6	<-22	4.6	140.8	34.5	12.5	8	7	-36	-44	15	70	80

TABLE 31

Perfusion		Type of Blood	T-Wave					Q-T Interval					Rate				
			Cont.	30"	60"	Ch. 30"	Ch. 60"	Cont.	30"	60"	Ch. 30"	Ch. 60"	Cont.	30"	60"	Ch. 30"	Ch. 60"
8	8 (4)	ACD	-2	-1.5	-1.5	+.5	+.5	10	11.5	12	+1.5	+ 2	87	77	61	- 10	- 26
	8 (5)	ACD	-3	-4	-3.5	- 1	-.5	12	12.5	12.5	+ .5	+.5	66	66	66	0	0
	8 (6)	ACD	-3.5	-3.5	-4	0	-.5	11	12	14	+ 1	+ 3	80	78	56	- 2	- 24
	DOG IV																
	8 (7)	ACD	-4	E.S. -3.5	E.S. ♥4.5	+.5	-.5	11.5	12.5	12	+ 1	+.5	71	90	80	+ 19	+ 9
	8 (8)	ACD	E.S.										50	50	46	0	- 4
	8 (9)	ACD	-3	-3	-4	0	-1	14	16	16	+ 2	+ 2	48	43	72	- 5	+ 24
	DOG V																
	8 (10)	H	E.S.										80	80	62	0	- 18
	8 (11)	H	-5	-3	-2	+2	+3	14	13	13	- 1	- 1	84	45	46	- 39	- 38
9	DOG I																
	9 (1)	H	-4	-3.5	-3.5	+.5	+.5	6.5	7	6.5	+.5	0	120	100	96	- 20	- 24
	9 (2)	ACD	-4	-4	-5	0	- 1	7	7	7.5	0	+.5	106	94	84	- 12	- 22

TABLE 32

PERFUSION	TYPE OF BLOOD	TEMP.	ADD. Ca	pH	PCO_2	STA. BIC.	B.E.	K	Na	Ca	V.C.F						B. P.	
											CONT.	30"	60"	%CH. 30"	%CH. 60"	REC.	CONT.	REC.
9 DOG II		37	--	7.37	23	16.5	-9.5	5	170	5.3								
DOG III		37		7.41	25.5	22.5	- 1	4.5	160	5.3								
9 (3)	ACD	37	.6	6.3	150	6	-22	6.7	147.5	34.5	15	17	17	+13	+13	16	100	90
9 (4)	ACD	10	.6	6.7	ca 40	6	-22	4.6	140.8	34.5	15	17	15	+13	0	16	100	100
9 (5)	ACD	40	.6	6.3	150	6	-22	4.6	140.8	34.5	17	16	14	- 6	-18	12	90	100
DOG IV		37		7.3	29.5	15.5	-10.5	3.6	156.3	6.2								
9 (6)	ACD	37	.6	6.3	150	6	-22	7	142.5	34.5	15	5	3	-67	-80	12	60	70
DOG V		37		7.38	30.5	19	- 5.5	3.4	160	6.2								
9 (7)	ACD	20	.6	6.55	ca 70	6	-22	4.6	140.8	34.5	14.5	5	3	-66	-80	13.5	70	60
9 (8)	ACD	10	.6	6.7	ca 40	6	-22	4.6	140.8	34.5	13	14	2	-69	-85	11.5	60	45
9 (9)	H	35	0	7.24	31	14	-12.5	3.4	160	6.2	8.5	9	10	+ 6	+18	9.5	50	50
9 (10)	H	35	0	7.24	31	14	-12.5	6	160	6.2	9	6	6	-33	-33	9	110	110
DOG VI		37		7.24	30.5	14	-12.5	3.4	160	6.2								

TABLE 33

Perfusion	Type of Blood	T - Wave					Q-T Interval					Rate.				
		Cont.	30"	60"	Ch 30"	Ch 60"	Cont.	30"	60"	Ch. 30"	Ch 60"	Cont.	30"	60"	Ch. 30"	Ch. 60"
9 DOG II	ACD															
DOG III																
9 (3)	ACD	-5	+1	0	+6	+5	6.5	6.5	7	0	+ .5	84	120	120	+ 36	+ 36
9 (4)	ACD	-4	+4	-3	+8	+1	6.5	8.5	8.5	+2	+2	94	100	90	+ 6	- 4
9 (5)	ACD	3	0	+4	-3	+1	8.6	6	7	-2.5	-1.5	130	120	120	- 10	- 10
DOG IV																
9 (6)		0	-2	-4	-2	-4	6.5	7	8	+ .5	+1.5	120	96	78	- 24	- 42
DOG V																
9 (7)	ACD	0	-2	-4	-2	-4	6.5	8	9	+1.5	+2.5	120	94	73	- 26	- 47
9 (8)	ACD	-1	-4	-5	-3	-4	7	11	12	+4	+5	115	76	66	- 39	- 49
9 (9)	H	-2	-2	-1.5	0	+.5	8	8.5	8	+.5	0	94	92	90	- 2	- 4
9 (10)	H	-2	-4	-4	-2	-2	7	8	8.5	+1	+1.5	100	80	75	- 20	- 25
DOG VI																

TABLE 24

PERFUSION	Type of Blood	TEMP	Add Ca	pH	PCO_2	STA. BIC.	B.E.	K	Na	Ca	V. C. F. CONT	30"	60"	% CH 30"	% CH 60"	REC	B. P CONT.	REC
9																		
9 (11)	H	10	0	7.65	ca 15	14	-12.5	3.4	160	6.2	9	9	9.5	0	+ 6	9	110	110
9 (12)	ACD	37	0	6.3	> 150	< 6	<-22	4.6	140.8	3.7	9	2	0.5	-77	-94	8	110	84
11 DOG I		37	0	7.37	34	21.5	- 3	3.1	137.5	5								
11 (1)	H	37	0	7.37	34	21.5	- 3	3.1	137.5	5	14.5	18°	17 A 20 L.T.	+24	+28	14.5	120	120
DOG II		37		7.48	34	25.5	+ 4	3.1	137.5	5								
11 (2)	ACD	37	.6	6.6	> 150	< 6	<-22	3.1	147.5	30	16.5	14 A 18 L.T.	15.5 A 18 L.T.	- 3	+ 3	15	120	115
11 (3)	ACD	37	.4	6.6	> 150	< 6	<-22	3.1	147.5	25.1	14.5	2.5	1.5	-84	-90	18	60	50
11 (4)	ACD	37	.6	7.05	> 150	28	7	2.7	180	30.9	18.5	8	4	-57	-78	18	55	70
11 (5)	ACD	37	.6	6.78	25	< 6	<-22	2.9	150.5	30.9	19	20 A 24 L.T.	24	+16	+26	18.5	45	60
DOG III		34°C		7.39	30	17.5	- 8											
11 (6)	ACD	37	.6	6.6	> 150	< 6	<-22	3.1	147.5	30	17.5	11 A 25 L.T.	E.S. 23	+ 3	+31	17.5	50	50
DOG IV		37		7.37	29	18.5	- 6											
11 (7)	H	37	0	7.37	29	18.5	-6	3.0	149	4.5	16	19 A 21 L.T.	24 A 26 L.T.	+25	+55	17	50	50

TABLE 35

Perfusion		Type of Blood	T-Wave					Q-T Interval					Rate				
			Cont	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"
9	9 (11)	H	-3	- 4	-7	-1	-4	8.5	11	12	+2.5	+3.5	84	50	63	- 34	- 21
	9 (12)	ACD	-4	- 6	-9	-2	-5	8.5	11	14	+2.5	+5.5	86	65	62	- 21	- 24
11	DOG I																
	11 (1)	H	+.5	+.5	-5	0	-1	7.5	7.5	7.5	0	0	112	128	125	+ 16	+ 13
	DOG II																
	11 (2)	ACD	-.5	0	+5 Alt. -1 Alt.	+.5	+5.5 Alt. - .5 Alt.	7.5	7	7.5	-.5	0	125	130	120	+ 5	- 5
	11 (3)	ACD	-.5	+ 2	+1.5	+2.5	+2	7.5	10	12	+2.5	+4.5	128	98	82	- 30	- 46
	11 (4)	ACD	+1.5	+ 2	+1.5	+ .5	0	7	7.5	8.5	+ .5	+1.5	130	126	118	- 4	- 12
	11 (5)	ACD	+1.5	0 Alt. + 1 Alt.	E.S.	- .1	0	7.5	7	E.S. 7	- .5	- .5	130	130	135	0	+ 5
	DOG III																
	11 (6)	ACD	+1	0	+5 Alt. 0 Alt.	- 1	+4 Alt. -1 Alt.	7	7.5	8	+ .5	+1	132	110	125	- 22	- 7
	DOG IV																
	11 (7)	H	+1	+.5	0	- .5	-1	7.5	7.5	7	0	-.5	128	130	125	+ 2	- 3

TABLE 36

	PERFUSION	Type of Blood	TEMP	Add Ca	pH	Pco_2	STA. BIC.	B.E.	K.	Na	Ca	V. C. F. CONT	V. C. F. 30"	V. C. F. 60"	V. C. F. % CH 30"	V. C. F. % CH 60"	V. C. F. REC	B. P. CONT	B. P. REC
11																			
	11 (8)	ACD	37	.6	<6.6	>150	< 6	<-22	7.15	145	30	18	17.5	16 A / 25 T	- 3	+ 14	17.5	70	60
	DOG V		33°C		7.35	26	15.5	-10											
	11 (9)	H	37	0	7.28	32	15.5	-10	6.9	137.5	3.9	17	21	24	+24	+ 41	17	45	45
	11 (10)	ACD	37	0	<6.6	>150	< 6	<-22	3.1	147.5	9.2	17	1	0	-95	-100	0	45	0
12	DOG I		37		7.45	23	19	- 5	2.4	145	5.1								
	12 (1)	ACD	37	.6	6.3	>150	< 6	<-22	2.7	155	25.4	14.5	10.5	11	-28	- 24	21	68	112
	12 (2)	ACD	37	.6	7.00	>150	32	+ 8	2.5	217	25.0	24.9	5	3	-80	- 88	33	70	72
	DOG II		36°C		7.43	21	17.5	- 7.5											
	12 (3)	ACD	37	.6	7.35	>150	>55	>+33	2.4	275	24.6	18	7	10	-61	- 44	31	84	76
	12 (4)	ACD	37	.6	7.40	50	27	+ 6	2.7	215	25.2	12	4	4	-67	- 67	17	44	66
	12 (5)	ACD	37	.6	6.25	>150	< 6	<-22	2.7	155	25.4	13.5	4.5	5.5	-67	- 59	24	40	66
	12 (6)	H	37	0	7.45	23	19	- 5	4.0	145	5.00	13	9	9	-31	- 31	9	48	50
	DOG III		34	---	7.29	17	10	-17											

TABLE 37

Perfusion	Type of Blood	T-Wave					Q-T Interval					Rate				
		Cont.	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"
11																
11 (8)	ACD	+2	+1.5	+2	- .5	0	7	7.5	7.5	+ .5	+ .5	130	130	130	0	- 0
DOG V																
11 (9)	H	+2	+1.5	+1	- .5	-1	7	7.5	7.5	+ .5	+ .5	130	132	136	+ 2	+ 6
11 (10)	ACD	+2	0	-4	-2	-6	7.7	8.5	14	+3	+6.5	130	90	78	-40	-52
12																
DOG I																
12 (1)	ACD	-7	-5.5	-6.5	+1.5	+.5	7.5	8	7.5	+ .5	0	145	135	130	-10	-15
12 (2)	ACD	-3.5	-4.5	+1	-1	+4.5	7	8.5	9	+1.5	+2	140	125	94	-15	-46
DOG II																
12 (3)	ACD	-4	-4	+2	0	+6	7.5	9	10	+1.5	+2.5	130	96	92	-34	-38
12 (4)	ACD	-2.5	-3.5	-3	-1	-.5	7	9	9	+2	+2	135	100	100	-35	-35
12 (5)	ACD	-4	irreg.	irreg.	-	-	7.5	7.5	7.5	-	-	130	irreg.	130	-	0
12 (6)	H	-6	irreg.	-6	-	0	7.5	irreg.	8	-	+ .5	125	100	132	-25	+ 7
DOG III																

TABLE 38

PERFUSION	Type of Blood	TEMP	Add Ca	pH	P_{CO_2}	STA BIC	B.E.	K	Na	Ca	V. C. F. CONT	30"	60"	% CH 30"	% CH 60"	REC	B. P. CONT	REC
12 12 (7)	ACD	37	.6	7.81	100	>60	>+30	2.5	247	25.2	3	4	3	+33	0	10	10	30
13 DOG I		37		7.46	25.5	21	- 3	2.8	150	4.5								
13 (1)	H	37	0	7.30	46	21	- 3	4.35	147.5	5.4	18.5	12	9	-35	-51	21.5	120	116
13 (2)	H	10	0	7.70	17	21	- 3	4.35	147.5	5.4	28	25	30	-11	+ 8	30	144	136
13 (3)	ACD	37	.7	6.4	>150	< 6	<-22	2.55	140	28.1	25	16	10	-36	-60	33	124	150
13 (4)	ACD	10	.7	6.81	ca40	< 6	<-22	2.60	165	38.4	26.5	15	15	-43	-43	36	100	130
13 (5)	ACD	20	.7	6.66	77	< 6	<-22	2.65	160	45.8	22.5	9	9	-60	-60	33	90	125
DOG II		31		7.44	33	20	- 3.5	2.20	130	5.8								
13 (6)	H	37	0	7.3	46	21	- 3	9.60	140	4.8	26	21	24	-19	- 8	22	95	100
13 (7)	H	37	0	7.3	46	21	- 3	11.50	135	3.2	24	20	12	-17	-50	22	100	88
13 (8)	ACD	37	.7	6.4	>150	< 6	<-22	7.55	155	43.4	27	15	9	-44	-67	30	100	120
13 (9)	ACD	37	.7	6.4	>150	< 6	<-22	11.0	132.5	37.8	28	23	22	-18	-21	28	110	120

TABLE 22

Perfusion	Type of Blood	T-Wave					Q-T Interval					Rate				
		Cont	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"	Cont	30"	60"	CH 30"	CH 60"
12 12 (7)	ACD															
13 DOG I																
13 (1)	H	-8	-11	- 6	-3	+2	7.5	7.5	7	0	- .5	105	110	110	+ 5	+ 5
13 (2)	H	-5.5	- 5	- 7	+.5	-1.5	7.5	8	9.5	+.5	+2	105	105	105	0	0.
13 (3)	ACD	-5	- 8.5	- 8	-3.5	-3	8	8	8	0	0	110	100	100	-10	-10
13 (4)	ACD	-6.5	0	- 6	+6.5	+ .5	8	—	10	—	+2	110	100	100	-10	-10
13 (5)	ACD	-6.5	- 5	-10	+1.5	-3.5	8	9	9.5	+ 1	+1.5	110	110	110	0	0
DOG II																
13 (6)	H	-7	- 4	- 4	+3	+3	7.5	6.5	7	- 1	- .5	100	130	125	+30	+25
13 (7)	H	-9	-11	-10	-2	-1	7	7	6	0	-1	135	140	130	+ 5	- 5
13 (8)	ACD	-8	-10	-12	-2	-4	6	6	6	0	0	150	135	135	-15	-15
13 (9)	ACD	-10	- 9	-11	+1	-1	6	6	6	0	0	160	150	160	-10	0

TABLE 40

	Perfusion	Type of Blood	Temp	Add Ca	pH	Pco_2	STA. BIC.	B. E.	K	Na	Ca	V. C. F. CONT.	V. C. F. 30"	V. C. F. 60"	V. C. F. % CH 30"	V. C. F. % CH 60"	B. P. CONT.	B. P. REC.
13	13 (10)	ACD	10	.7	6.81	ca 40	<6	<-22	2.55	140	40	24	31	34	+ 29	+42	100	80
	13 (11)	ACD	20	1.6	6.66	75	<6	<-22	2.55	140	60	7	28	33	+300	+371	40	—
	13 (12)	ACD	37	0	6.4	150	<6	<-22	2.55	140	3.1	12	4	2.5	- 67	- 80	50	0
14	DOG I		36		7.38	29	18.5	- 6.5	2.75	138.75	5.9							
	14 (1)	H	40	0	7.33	41	20.8	- 3	3.7	140	4.22	24	28	21	+ 17	- 13	120	136
	14 (2)	H	10	0	7.73	15	20.8	- 3	3.7	140	4.2	20	21	20	+ 5	0	128	128
	DOG II		35		7.38	29	19.5	- 4.5										
	14 (3)	ACD	37	.7	6.45	> 150	<6	<-22	2.95	140	30	23	7	4	-69	- 83	100	108
	DOG III		34		7.40	31	19.5	- 4										
	14 (4)	ACD	10	.7	6.85	ca 40	<6	<-22	3	137.5	33.3	17	16	17	- 6	0	100	100
15	DOG I		37		7.42	36.5	23.5	+ 0.5	3.6	137.5	5.5							
	15 (1)	H	37	0	7.41	29.5	20.5	- 3.5	5.65	135	4.4	18	14	12	-22	- 33	120	112
	15 (2)	H	10	0	7.82	ca 12	20.5	- 3.5	5.5	135	4.4	17	12	18	-29	+ 6	116	110

TABLE 41

Perfusion	Type of Blood	V.C.F.	T-Wave					Q-T Interval					Rate				
		Rec.	Cont.	30"	60"	CH 30"	CH 60"	Cont.	30"	60"	CH 30"	CH 60"	Cont.	30"	60"	CH 30"	CH 60"
13 13 (10)	ACD	19	- 8	-5	-7	+3	+1	6	6.5	7.5	+.5	+1.5	160	150	150	- 10	- 10
13 (11)	ACD	13	-11	-4	-3	+7	+8	7.5	7	7	-.5	- .5	97	103	90	+ 6	- 7
13 (12)	ACD	0	-10	-6	+3	+4	+13	7	8	11	+1	+4	100	90	80	- 10	- 20
14 DOG I																	
14 (1)	H	24	- 2	-2	-4	0	-2	7.5	7.5	8	0	+ .5	96	100	100	+ 4	+ 4
14 (2)	H	20	- 3	-2	-1.5	+1	+1.5	7	7	7.5	0	+ .5	112	125	100	+ 13	- 12
DOG II																	
14 (3)	ACD	23	- 5	-8	-11	-3	-6	7	7.5	7.5	+.5	+ .5	130	115	110	- 15	- 20
DOG III																	
14 (4)	ACD	20	- 6	-4	- 6	+2	0	6.5	7	8	+.5	+1.5	135	106	104	- 29	- 31
15 DOG I																	
15 (1)	H	18	- 1	-1	-2.5	0	-1.5	8	8	8	O	O	106	96	92	- 10	- 14
15 (2)	H	17	- 2	-1.5	- 3	+.5	-1	8	9	10	+ 1	+2	98	88	80	- 10	- 18

TABLE 42

PERFUSION	Type of Blood	TEMP	Add Ca	pH	PCO_2	STA. BIC.	B.E.	K	Na	Ca	V. C. F. CONT.	V. C. F. 30"	V. C. F. 60"	V. C. F. % CH 30"	V. C. F. % CH 60"	B. P. CONT	B. P. REC.
15 15 (3)	ACD	37	.7	6.45	125	< 6	<-22	3.30	141	38.8	16	9.5	9	-41	-44	116	124
15 (4)	ACD	10	.7	6.84	ca 39	< 6	<-22	3.30	140	33.5	16	12	11	-25	-31	116	124
15 (5)	ACD	37	.7	6.45	125	< 6	<-22	6.5	140	36.0	16	21	19	+31	+18	116	150
15 (6)	H	37	0	7.41	29.5	20.5	- 3.5	11.9	140	3.6	18.5	17.5	11	- 5	-45	120	100
DOG II		36		7.40	34	21.5	- 1.5	3.85	142.5	6.2							
15 (7)	H	37	0	7.41	29.5	20.5	- 3.5	14.45	135	3.9	18.5	13	7 9 AFT	-30	-57	120	108
15 (8)	ACD	10	.4	6.86	ca 40	< 6	<-22	3.00	132.5	27.0	15.5	6	15	-61	- 3	136	140
15 (9)	ACD	37	.4	6.40	125	< 6	<-22	3.40	145	28.4	16.5	6	5	-58	-70	140	130

TABLE 43

PERFUSION	Type of Blood	V.C.F.	T- WAVE					Q-T INTERVAL					RATE				
		REC.	CONT	30"	60"	CH 30"	CH 60"	CONT	30"	60"	CH 30"	CH 60"	CONT	30"	60"	CH 30"	CH 60"
15 15 (3)	ACD	16.5	-2	-2	-1.5	0	+ .5	8.5	8.5	8.5	0	0	100	93	88	- 7	-12
15 (4)	ACD	16	-2	-2	-1	0	+1	8.5	8.5	8.5	0	0	94	92	88	- 2	- 6
15 (5)	ACD	19	-2	+2	+1	+4	+3	7.5	7	7.5	-.5	0	115	113	115	- 2	0
15 (6)	H	19	-2	-2.5	-4.5	-.5	-2.5	7.5	6.5	6.5	- 1	-1	115	115	84	0	-31
DOG II																	
15 (7)	H	15	-2	-3	-3	-1	-1	7	7	7.5	0	+.5	125	112	88	-13	-37
15 (8)	ACD	19	-2.5	-9	-7.5	-6.5	-5	7	9	9	+2	+2	120	115	98	- 5	-22
15 (9)	ACD	18	-2	-5.5	-5.5	- 3.5	-3.5	6.5	7.5	7.5	+1	+1	130	115	105	-15	-25

REFERENCES

Abbott, J.P., Cooley, D.A., De Bakey, M.E., Ragland, J.E., Storage of Blood for Open Heart Operations: Experimental and Chemical Observations. Surg. 44, 698, 1958.

Adams, W.E., Thornton, T.F. Jr., Allen J.G., Gonzalez, D.E., The Danger and Prevention of Citrate Intoxication in Massive Transfusions of Whole Blood. Ann. Surg. 120, 656, 1944.

Adamsons, K.Jr., Daniel S.S., Gaudy, G., James L.S., Influence of Temperature on Blood pH of the Human Adult and Newborn. J. Appl. Physiol. 19, 897, 1964.

Agote L., Naevo Procidimento para le Transfusion del Sangre. Ann. Inst. Chin. Med., B. Air., Jan. 1915.

Ames, R., Syllm, I., Rapoport, S., Effect of Infusions of Citrated Plasma on the Plasma Citrate Level of Infants. Paediatrics 6, 361, 1950.

Andersen, O.S., Sampling and Storing of Blood for Determination of Acid-Base Status. Scand. J. Clin. Lab. Invest., 13, 196, 1961.

Andersen,O.S., Engel, K., A New Acid-Base Nomogram. Scand. J. Clin. Lab. Invest. 12, 177, 1960.

Andersen, O.S., Engel, K., Jorgenson, K., Astrup, P., A Micro Method for Determination of PH Carbon Dioxidetnsion, Base Excess and Standard Bicarbonate in capillary blood. Scand. J. Lab. Clin. Invest. 12, 172, 1960.Ast

Astrup, P., A Simple Electrometric Technique for the Determination of Carbon Dioxidetension in Blood and Plasma, Total content of carbon dioxide in plasma and bicarbonate content in "separated"plasma at a fixed Carbon DioxideTension (40 mm. Hg.). Scand. J. Clin. Lab. Invest. 8, 33, 1956.

Astrup, P., Jorgensen, K., Andersen, O.S., Engel, K., The Acid-Base Metabolism. A New Approach. Lancet I, 1035, 1960.

Austin, W.H., Lacombe, E.H., Rand, P.W., Chatterjee, M., Solubility of Carbon Dioxide in Serum from 15 to 38°C. J. Appl. Phys. 18, 301, 1963.

Baue, A.E., Hermann, G., Shaw, R.S., Study of Bank Blood Toxicity. S.G. & O. 113, 40, 1961.

Baron, D.N., Bell, J.L., Complexiметric Determination of Calcium in Pathological and Physiological Specimens. J. Clin. Path. 12. 143, 1959.

Bechtel, J.T., White, J.E., Estes, E.H.Jr., The Electrocardiographic Effects of Hypocalcemia Induced in Normal Subjects with Edathamil Disodium. Circulation 13, 837, 1956.

Beer, R., Stoffwechselveränderungen während des Extrakorporalen Kreislaufs. Thorax Chirurgie 6, 360, 1959.

Bell, G.H., Davidson, J.N., Scarborough, H., Text Book of Physiology and Biochemistry (5th Edition). E & S Livingstone 1961.

Bett, I.M., Fraser, G.P., A rapid micro-method for determining serum calcium/

calcium. Clin. Chim. Acta. 4, 346, 1959.

Blair, E., Montgomery, A.V., Swan, H., Posthypothermic Circulatory Failure. I Physiologic Observations on the Circulation. Circulation 13, 909, 1956.

Boyan, C.P., Howland, W.S., Blood Temperature; A critical Factor in Massive Transfusions. Anaesthesiology 22, 559, 1961.

Boniface, K.J., Brodie, O.J., and Walton, R.P., Resistance Strain Gauge arches for direct Measurement of Heart Contractile Force in Animals. Proc. Soc. Exper. Biol. and Med. 84, 263-266; 1953.

Boyan, C.P., Howland, W.S., Problems Related to Massive Blood Replacement. Anaesth. Analg. 41, 497, 1962.

Boyan,C.P., Cold or Warmed Blood for Massive Transfusions. Ann. Surg. 160, 282, 1964.

Bradley, A.F., Stupfel, M., and Severinghaus, J.W., Effects of Temperature on PCO_2 and PO_2 of Blood in Vitro. J. Appl. Physiol. 9, 201, 1956.

Brewin, E.G., Gauld, R.P., Nashat, F.S., Neil, E., An Investigation of problems of Acid-Base Equilibrium in Hypothermia. Guy's Hospital Repprts 104, 177-213, 1955.

Brooks, D.K., Physiological Responses Produced by Changes in Acid-Base Equilibrium following Profound Hypothermia. Anaesthesia 20(2), 173, 1965.

Bruneau, J. and Graham, E.A., A Caution against Too Liberal Use of Citrated Blood in Transfusions. Arch. Surg. 47, 319, 1943.

Bücherl, E.S., Horkenback, G., Schmutzer, K.J., Probleme des Saure-Basenhaushalter Während Extracorporaler Zirkulation. Thorax Chirurgie 6, 505, 1959.

Bunker, J.P., Stetson, J.B., Coe, R.C., Grillo, H.C. and Murphy, A.J., Citric Acid Intoxication. J.A.M.A. 157, 1361, 1955.

Bunker, J.P., Citric Acid Intoxication. Proc. 6th Cong. of Inst. Soc. of Blood Transfus. p69, 1956.

Burnell, J.M., Villamil, M.F., Uyeno, B.T., Scribner, B.H., The Effect in Humans of Extracellular pH Change on the Relationship Between Serum Potassium Concentration and Intracellular Potassium. J.Clin. Invest. 35 (pt. 2), 935, 1956.

Carrel, A., La Technique Opératoire des Anastomoses Vasculaires et la Transplantation des Visceres. Lyon Med. 98, 859, 1902.

Chang, T.S., Freeman, S., Citric Acid and its relation to serum and Urinary Calcium. Amm. J. Physiol. 160, 330-334, 1950.

Chollet, H.A., Clauss, R.H., Gianninni, S.G., MacFee, W.F., A Safe and Simple Method of Blood Collection for Transfusion Utilizing and Cation Exchange/

Exchange Resin. Ann. Surg. 147, 69, 1958.

Clarke, N.E., The Action of Calcium on the Human Electrocardiogram. Ann. Heart J. 22, 367, 1941.

Clowes, G.H.A. Jr., Simeone, F.A., Acute Hypocalcemia in Surgical Patients. Ann. Surg. 146, 530, 1957.

Clowes, G.H.A., Neville, W.E., Sabga, G., Shibota, Y., The Relationship of oxygen consumption, perfusion rate, and temperature to the acidosis associated with cardiopulmonary circulatory bypass. Surgery 44, 220, 1958.

Cookson, B.A., Costas-Durieux, J., Bailey, C.P., Toxic Effects of Citrated Blood and the Search for a Suitable Substitute for Use in Cardiac Surgery. Ann. Surg. 139, 430-438, 1954.

Corsa, L. Jr., Olney, J.M., Steenburg, R.W., Ball, M.R., Moore, F.D., The Measurement of Exchangeable Potassium in Man by Isotope Dilution. J. Clin. Invest. 29, 1280, 1950.

Cotten, M.deV., Circulatory changes Affecting Measurement of Heart Force in Situ with Strain Gauge Arches. Am. J. Physiol. 174, 365, 1953.

Cotten, M. deV., Bay, E., Direct Measurement of Changes in Cardiac Contractile Force: Relationship of Such Measurement to Stroke Work Isometric Pressure Gradient and other Parameters of Cardiac Function. Am. J. Physiol. 187, 122, 1956.

Cotten, M. deV., Maling, M., Relationships Among Stroke Work, Contractile Force and Fiber Length during Changes in Ventricular Function. Am. J. Physiol. 189(3); 580-586, 1957.

Darby, T.D., Aldinger, E.A., Lee, W.H.Jr., Tenney, J.M., Evaluation of Factors Affecting Cardiac Function during Open Heart Surgery. Federation Proc. 17, 362, March, 1958.

Darby T.D., Aldinger, E.E., Gadsden, R.H., Thrower, W.B., Effects of Metabolic Acidosis on Ventricular Isometric Systolic Tension and the Response to Epinephrine and Levarterenol. Circul. Res. 8, 1242, 1960.

Darby, T.D., Parker, E.F., Lee, W.H.Jr., Ashmore, J.D., The Influence of Cardio Pulmonary Bypass with Cardiac Arrest and Right Ventriculotomy on Myocardial Contractile Force. Ann. Surg. 147 (5), 596-602, 1958.

Duliere, W.L., La Teneur en Potassium et l'age d'un Serum. Compt. Rend. Soc. Biol. 107, 261, 1931.

Emanuel, D.A., Scott, J.B., Haddy, F.J., Effect of Potassium on Small and Large Blood Vessels of the Dog Forelimb. Am. J. Physiol. 197, 637, 1959.

Epstein, R.M., Nahas, G.G., Mark, L.C., Circulatory changes following rapid correction of severe hypereapnic acidosis by 2amime-2hydoxymethyl-1-3-Propanediol. Ann. N.Y. Acad. Sci. 92, 500, 1961.

Firt, P., and Hejhal, L., Sodium Citrate; one of the main causes of Cardiac Overloading and Failure in Rapid Blood Transfusions. Rev. Czechoslov. Med.

I/

1, 20-42, 1955.

Firt, P. and Hejhal, L., Concerning the Failure of the Present Treatment of Severe Haemorrhage and a New Method of Rapid Intravenous Transfusion. Proc. 6th Cong. of Int. Soc. of Blood Transfus. p86, 1956.

Firt, P., and Hejhal, L., Treatment of Severe Haemorrhage. Lancet 273, 1132, 1957.

Fleishman, M., Scott, J.B., Haddy, F.J., Effect of pH Change upon Systemic Large and Small Vessel Resistance. Circulation Res. 5, 602, 1957.

Flink, E., Watson, C., A Method for the Quantitative Determination of Haemoglobin and Related Heme Pigments in Feces, Urine and Blood Plasma. J. Biol. Chem. 146, 171, 1942.

Foote, A.V., Trede, M., Maloney, J.V., Jr., The Use of Stored Acid-Citrate-Dextrose (ACD) Blood for Clinical Extracorporeal Circulation. Surg. For. 11, 230, 1960.

Foote, A.V., Trede, M., Maloney, J.V., Jr., An experimental and Clinical Study of the Use of Acid-Citrate-Dextrose (ACD) Blood for Extracorporeal Circulation. J. Thor. Card. Surg. 42, 93-109, 1961.

Gain, E.A., The Problem of Cardiac Collapse Associated with the Massive Transfusion of Citrated Blood. Canad. Anaesth. Soc. J. 9, 207, 1962.

Geszti O., Ts'Ao Wei Chi and Li Tien Huang, Experimental Data about the Heparin Neutralising Effect of Intravenously Administered Calcium. Acta. Physiologica. Hungarica 13, 341-354, 1958.

Gibson, J.G. II, Rees, S.B., McManus, T.J., Scheitlin, W.A., A citrate-Phosphate-Dextrose Solution for the Preservation of Human Blood. Amer. J. Clin. Path. 28, 569, 1957.

Gibson, J.G., Blood Component Therapy. Bull. South Central Assoc. of Blood Banks. 3-12, Dec. 1959.

Golden, J.S., Brams, W.A., Mechanism of the Toxic Effects from Combined Use of Calcium and Digitalis. Ann. Int. Med. 11, 1084, 1938.

Haddy, F.J., Scott, J.B., Florio, M.A., Daugherty, R.M., Jr., Huinzenga, J. N., Local Vascular Effects of Hypokalaemia, Alkadosis, Hypercalcaemia and Hypomagnesemia. Ann. J. Physiol. 204, 202, 1963.

Hara, M., Doherty, J.E., Williams, G.D., Citric Acid Metabolisms in the Hypothermic Dog. Surgery 49, 734, 1961.

Harkins, H.N., and Hastings, A.B., Study of Electrolyte Equilibrium in Blood in Experimental Acidosis. J. Biol. Chem. 90, 565, 1931.

Hastings, A.B., McLean, F.C., Eichelberger, L., Hall, J.L., Da Costa, E., J. Biol. Chem. 107, 351, 1934. The Ionisation of Calcium, Magnesium and Strontium Citrates.

Hoff, H.E., Smith, P.K., Winkler, A.W., Electrocardiographic Changes and Concentration of Calcium in Serum Following Intravenous Injection of Calcium/

Calcium Chloride. Am. J. Physiol. 125, 162, 1939.

Howland, W.S., Schweizer, O., Boyan, C.P., Dotto, A.C., Physiologic Alterations with Massive Blood Replacement. S. G. and O, 101, 478-482, 1955.

Howland, W.S., Boyan, C.P., Schweizer, O., Ventricular Fibrillation during Massive Blood Replacement. Am. J. Surg. 92, 356, 1956.

Howland, W.S., Bellville, J.N., Zucker, M.B., Boyan, C.P., Clifton, E.E., Massive Blood Replacement, V Failure to observe Citrate Intoxication. Surg. Gyn. and Obst. 105, 529, 1957.

Howland, W.S., Jacobs, R.G., Goulet, A.H., An Evaluation of Calcium Administration During Rapid Blood Replacement. Anesth. Analg. 39, 557, 1960.

Howland, W.S., and Schweizer, O., Acid-Base Balance during shock and Blood Transfusions. Anaesth. Analg. 41, 634-638, 1962.

Howland, W.S., Schweizer, O., The Use of "Standard Bicarbonate" in Differentiating Disorders of Acid-Base Balance. Anaesth. Analg. 42, 416, 1963.

Hubbard, T.F., Neis, D.D., Barmore, J.L., Severe Citrate Intoxication During Cardiovascular Surgery. JAMA 162, 1534, 1956.

Hustin, A., Principe d'une Nouvelle Methode de Transfusion Muqueuse. J. Med.Brux 12, 436, 1914.

Ivy, A.C., Greengard, H., Stein, I.F., Gradins, F.S., and Dutton, D.F., The effects of Varions Blood-Substitutes in Resuscitation After an Otherwise Fatal Haemorrhage. Surg. Gynaec. and Obst. 76, 85, 1943.

Jorgensen, K., Astrup, P., Standard Bicarbonate, Its Clinical Significance and a New Method for its Determination. Scand. J. Clin and Lab. Invest. 9, 122, 1957.

Katz, L.N., Lindner, E., Action of Excess Na, Ca and K on coronary vessels. A.J. Physiol 124, 155, 1938.

Kingsley, G.R., Dowdell, L.A., Direct Iodometric Determination of Blood Chloride. J. Lab. Clin. Med. 35, 637, 1950.

Krumhaar, D., Foote, A.V., Maloney, J.V.Jr., Survival after Extracorporeal Circulation of Stored Erythrocytes Treated with Acid-Citrate-Dextrose (ACD) and Heparin. J. Surg. Res. 3 (8), 404-408, 1963.

Lee, W.H.Jr., Darby, T.D., Ashmore, J.D., Parker, E.F., Myocardial Contractile Force as a Measure of Cardiac Function during Cardiopulmonary Bypass Procedures. Surg. Forum 8, 398-402, 1957.

Le Veen, H.H., Schatman, B., Falk, G., Cardiac Arrest Produced by Massive Transfusions. S.G. and O 109, 502, 1959.

Lewis, L.L., Melnick, L.M., Determination of Calcium and Magnesium with Erythlenedinitrilo-tetracetic acid. Studies in Accuracy. Analytical Chemistry 32 (1), 38, Jan. 1960.

Lewisohn/

Lewisohn, R., Blood Transfusion by the Citrate Method. Surg. Gynae. and Obst. 21, 37, 1915.

Litwin, M.S., Smith, L.L., Moore, F.D., Metabolic Alkalosis following Massive Transfusion. Surgery 45 (5), 805-813, 1959.

Lobpreis, E.L., Raschke, E., Watanabe, Y., Maloney, J.V. Jr., A Clinical Evaluation of Fresh and Stored Heparinised Blood for Use in Extracorporeal Circulation. Ann. Surg. 152, 947, 1960.

Loutit, J.F., Mollison, R.L., Advantages of a Disodium-Citrate, Glucose Mixture as a Blood Preservative. B.M.J. (ii), 744, 1943.

Loutit, J.F., Mollison, R.L., Young, I.M., Citric acid-sodium-citrate-glucose mixtures for blood storage. Quart. J. Exp. Physiol. 32, 183, 1943.

Loutit, J.F., Factors influencing preservation of stored red cells. J. Path. Bact. 57, 325, 1945.

Love, G.R., Studies on the Pharmacology of Sodium Citrate. I. Circulation. J. Lab. and Clin. Med. 9, 175, 1923.

McElroy, W.T., Jr., Gerdes, A.J., Brown, E.B. Jr., Effects of CO_2 Bicarbonate & pH on the performance of isolated perfused guinea pig hearts. Am. J. Physiol. 195, (2), 412-16, 1958.

MacGovern, J.J., Bunker, J.P., Goldstein, R., Finkbiner, R.B., The Stability of Coagulation Factors in Stored Blood. Proc. VIIth Cong. of Int. Soc. for Blood Transfusion. 10, 784, 1959.

McLean, F.C., Hastings, A.B., Report on a Biological Method for the Esti-:mation of Calcium Ion Concentration. J. Biol. Chem. 107, 334, 1934.

McLean, F.C., Hastings, A.B., The State of Calcium in the Fluids of the Body. J. Biol. Chem. 108, 285, 1935.

Maloney, J.V. Jr., Personal communication. 1965.

Martensson, J., On the Citric Acid Metabolism in Mammals. Acta. Physiol. Scand. 1 Supp. (2), 94-96, 1940.

Mellemgaard, K., Astrup, P., The Quantitative Determination of Surplus Amounts of Acid or Base in the Human Body. Scand. J. Clin. Lab. Invest. 12, 187, 1960.

Melrose, D.G., Dreyer, B., Bentall, H.H., Baker, J.B.E., Elective Cardiac Arrest. Lancet 269, 21-22, 1955.

Miller, G., McCoord, A.B., Joos, H.A., Clausen, S.W., Studies of Serum Electrolyte Changes during Exchange Transfusions. Paediatrics, 13, 412 1954.

Mollison, P.L., Young, I.M., In Vivo survival in the Human Subject of Transfused Erythrocytes after Storage in Various Preservative Solutions. Quart. J. Exper. Physiol. 31, 359, 1942.

Moore/

Moore, F.D., Saunders, W.B., Metabolic Care of the Surgical Patient. 1959.

Nahas, G.G., Malm, J.R., Manger, W.M., Verosky, M., Sullivan, S.F., Control of Acidosis and the Use of Titrated ACD Blood in Open-Heart Surgery. Annals Surgery 160(6), 1049, 1964.

Nakasone, N., Watkins, E.Jr., Janaway, C.A., Gross, R.E., Experimental Studies of Circulatory Derangement following the Massive Transfusion of Citrated Blood. J. Lab. Clin. Med. 43, 184, 1954.

Nealon, T.F., Abnormal Electrolyte Concentrations in Bank Blood and their Removal Prior to Transfusion by Passage over Ion Exchange Resins. Surg. Clin. N.Amer. 40(6), 1591, 1960.

Neptune, W.B., Bougas, J.A., Panico, F.G., Open-Heart Surgery without the Need for Donor-Blood Priming in the Pump Oxygenator. New. Eng. J. Med. 263, 111, 1960.

Overbeck, H.W., Molnar, J.I., Haddy, F.J., Resistance to Blood Flow through the Vascular Bed of the Dog Forelimb. Local Effects of Sodium, Potassium Calcium, Magnesium, Acetate, Hypertonicity and Hypotonicity. Amn. J. Cardiol 8, 533, 1961.

Paton, B.C., Rosenkrantz, J.G., Blount, S.G.Jr., Clinical and Physiological Results of Perfusion with Diluted Blood. Circulation 29, 63, 1964.

Peirce, E. Converse II, Huggin, P.M., Lee, R., A rapid quantitative Method of Determining Metabolic Respiratory pH Factors. Trans. Am. Soc. Artif. Int. Orgs. 5, 265, 1959.

Pepper, H., Lindsay, S., Responses of Platelets, Eosinophils, and Total Leucocytes During and Following Surgical Procedures. S.G. and O, 110, 319 1960.

Perkins, H.A., Osborn, J.J., Hurt, R., Gerbode, F., Neutralisation of Heparin in Vivo with Protamine, A Simple Method of Estimating the Required Dose. J. Lab. Clin. Med. 48, 223, 1956.

Reynolds, T.B., Martin, H.E., Homann, R.E., Serum Electrolytes and the Electrocardiogram. Amer. Heart J. 42, 671, 1951.

Ringer, S., A Further contribution regarding the influence of the Different Constituents of the Blood on the Contraction of the Heart. J. Physiol. 4, 29, 1883.

Roberts, K.E., Magida, M.G., Electrocardiographic Alterations Produced by a Decrease in Plasma pH Bicarbonate and Sodium as compared with those Produced by an Increase in Potassium. Circulation Research 1, 206-213, 1953.

Roebuck, M.J., Nahas, G.G., Mark, L.C., Habif, D.V., Prevention and Correction of Post-hypercapnic Arrhythmia with Sodium Chloride Administration. Surg. For. 13, 189, 1962.

Rosenthal, T.B., Effect of Temperature on pH of Blood and Plasma in Vitro/

Vitro. J. Biol. Chem. 173, 25, 1948.

Saffran, M., Denstedt, O.F., The effect of Intravenously Injected Citrate on the Serum Ionized Calcium in the Rabbit. Canad. J. Med. Sc. 29, 245, 1951.

Salant, W., Wise, L.E., The Action of SodiumCitrate and its Decomposition in the Body. J. Biol. Chem. 28, 27, 1916.

Schechter D.C., Swan, H., Biochemical Alterations of Preserved Blood. Results in two different citrate solutions (ACD and CPD). Arch. Surg. 84, 269, 1962.

Schechter, D.C., Paton, B.C., Swan, H., Extracellular Changes in Stored Blood. A Comparison of changes in Blood Treated with ACD solution or Cation Exchange Resins. Arch. Surg. 84, 358, 1962.

Schenk, W.G., Delin, N.A., Camp Capt. F.A., McDonald, K.E., Pollock, L., Gage, A.A., Chardock, W.M., Assisted Circulation: An Experimental Evaluation of Counterpulsation and Left Ventricular Bypass. Arch. Surg. 88, 327-344, 1964.

Schwaizer, O., Howland, W.S., The Effect of Citrated Bank Blood on Acid-Base Balance. S.G. and O, 114, 80, 1962.

Scott, J.B.,Frohlich, E.D., Hardin, R.A., Haddy, F.J., Na, K, Ca and Mg ion action on coronary vascular resistance in the dog heart. Am. J. Physiol 201, 1095, 1961.

Scribner, B.H., Burnell, J.M., Interpretation of Serum Potassium Concentration. Metabolism, 5, 468, 1956.

Severinghaus, J.W., Stupfel, M., Bradley, A.F., Variations of Serum Carbonic Acid pK withpH and Temperature. J. Appl. Physiol. 9, 197, 1956.

Smith, P.K., Winkler, A.W., Hoff, H.E., Calcium and Digitalis Synergism: Toxicity of Calcium Salts injected intravenously into digitalized animals. Arch. Int. Med. 64, 322, 1939.

Smith, W.W., Brown, I.W., Young, W., Glenn Jr., Sealy, W.C., Studies of Edglugate-Mg: A New Donor Blood Anticoagulant-Preservative Mixture for Extracorporeal Circulation. J. Thorac. and Cardiovasc. Surg. 38, 573, 1959.

Smith, W.W., Brown, I.W. Jr., Eadie, G,S., Experience with Edglugate-Mg. Donor Blood for Extracorporeal Circulation and Ordinary Transfusion. Bibl. Haemat. 13, 95, 1962.

Sodi-Pallares, and Calder. New Bases of Electrocardiography. 1956.

Sollmann, T., Heparin: A Manual of Pharmacology : W.B. Saunders Co. Phila. and Lond. 1957.

Sprague, C.C., Shapleigh, J.B., Mayes, S., Lange, R.D., Moore, C.V., Post-Transfusion survival of Erythrocytes Stored in a Solution of Ethylene-diamine Tetracetic Acid and Dextrose. J. Lab. Clin. Med. 41, 84, 1953.

Stewart/

Stewart, H.J., Shepard, E.M., Herger, E.L., Electrocardiographic Manifestations of Potassium Intoxication. Am.J. Med. 5, 821, 1948.

Strumia, M.M., The Immediate Collection Damage to Red Cells. Proc. 6th Cong. of Int. Soc. of Blood. Transfus. 303, 1956.

Surawicz, B., MacDonald, M.G., Kaljot, V., Bettinger, J.C., Treatment of Cardiac Arrhythmias with Salts of Ethylenediamine tetracetic acid (EDTA) Amer. Heart J. 58, 493, 1959.

Thomson, N.B.Jr., Ehrlich, F., Heparin Metabolism as Related to Time and Temperature. J. Thor. Card. Surg. 48, 19, 1964.

Tisdall, F.F., A note on the Kramer-Tisdall Method for the Determination of Calcium in Small amounts of Serum. J. Biol. Chem. 56, 439, 1923.

Trede, M., Foote, A.V., Maloney, J.V.Jr., Pathophysiological Aspects of Profound Hypothermia with Extracorporeal Circulation. Ann. Surg. 154 (2), 210-224, 1961.

Valtis, D.J., Kennedy, A.C., Defective Gas Transport Function of Stored red blood cells. Lancet i, 119, 1954.

Wall, H.C., Measurement of Circulation Time with Calcium Gluconate in Patients Receiving Digitalis, with Electrocardiographic Studies. Amer. Heart J. 18, 228, 1939.

Walter, C.W., A New Technique for Collection, Storage and Administration of Unadulterated Whole Blood. Surg. Forum (1950), 1, 483, 1951.

Watkins, E.Jr., Experimental Citrate Intoxication During Massive Blood Transfusion. Surg. Forum IV, 213-219, 1953.

Waidner, M.G.Jr., Clowes, G.H.A.Jr., The Role of the Kidney in Hypocalcemia Induced by Citrate Infusion. Surg. Forum X, 444-449, 1959.

Wexler, I.B., Pincus, J.B., Matelson, S., Lugovoy, J.K., The Fate of Citrate in Erythroblastiotic Infants Treated with Exchange Transfusions. J. Clin. Invest. 28, 474, 1949.

Winkler, A.W., Hoff, H.E., Smith, P.K., Electrocardiographic Changes and Concentration of Potassium in Serum following Intravenous Injection of Potassium Chloride. Amer. J. Physiol. 124, 478, 1938.

Winkler, A.W., Hoff, H.E., Potassium and the Cause of Death in Traumatic Shock. Amer. J. Physiol. 139, 686, 1943.

Woolen, J.W., Walker, P.G., An improved flame-photometric method for determination of Calcium in Serum. J. Clin. Path. 12, 149,1959.

Wurzel, H.A., Kirby, C.K., Moltham, M., Variations in Coagulation Factors Associated with Extracorporeal Circulation. Proc. VIIth Cong. of Int. Soc. of Blood Transfusion 10, 32, 1959.

von Ziemssen, H., Ueber Transfusion, Münch. Med. Wschr. 41, 349, 1894.

Zuhdi, N., McCullough, B., Carey, J., Greer, A., The Use of Citrated Banked

Banked Blood for Open Heart Surgery. Anaesthesiology 21, 496, 1960.

Zwemer, R.L., Scudder, J., Blood Potassium during Experimental Shock. Surgery 4, 510, 1938.

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